



Europäisches Patentamt  
European Patent Office  
Office européen des brevets



(11) Publication number:

**0 427 680 A1**

(12)

## EUROPEAN PATENT APPLICATION

(21) Application number: 90810854.1

(51) Int. Cl.<sup>5</sup>: **C07D 498/18, A61K 31/33,**  
///(C07D498/18,311:00,273:00,  
221:00)

(22) Date of filing: 07.11.90

(30) Priority: 09.11.89 DE 3937336  
16.11.89 DE 3938132  
23.12.89 DE 3942831  
23.12.89 DE 3942833  
05.03.90 DE 4006819

(43) Date of publication of application:  
15.05.91 Bulletin 91/20

(84) Designated Contracting States:  
AT BE CH DE DK ES FR GB GR IT LI LU NL SE

(71) Applicant: **SANDOZ LTD.**  
Lichtstrasse 35  
CH-4002 Basel(CH)

(84) BE CH DK ES FR GB GR IT LI LU NL SE

Applicant: **SANDOZ-PATENT-GMBH**

Humboldtstrasse 3  
W-7850 Lörrach(DE)

(84) DE

Applicant: **SANDOZ-ERFINDUNGEN**  
Verwaltungsgesellschaft m.b.H.  
Brunner Strasse 59  
A-1235 Wien(AT)

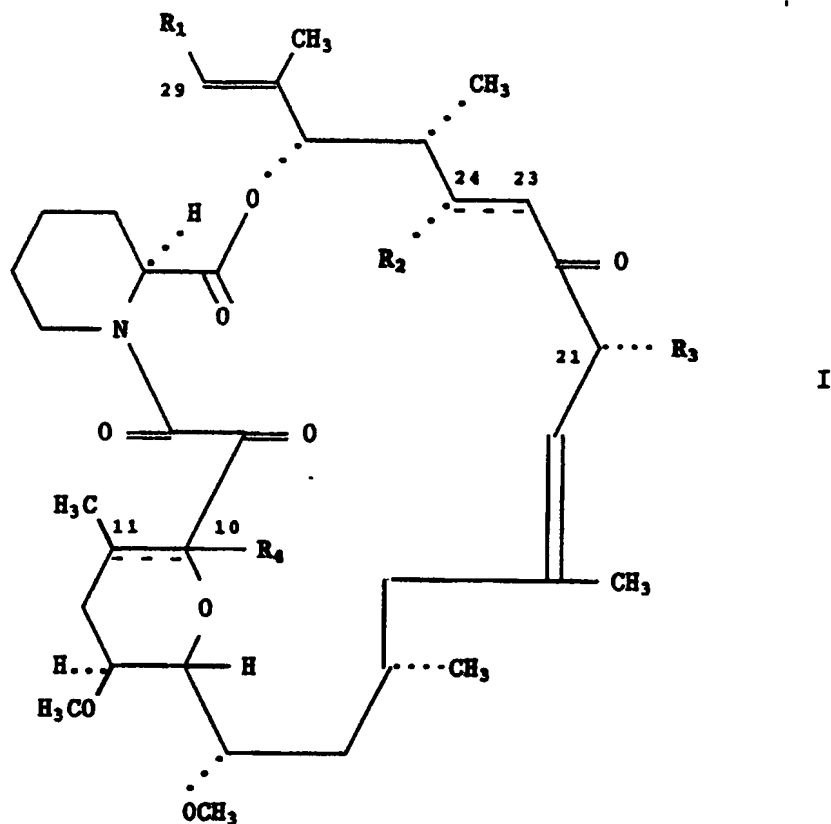
(84) AT

(72) Inventor: Baumann, Karl  
Simmeringer Hauptstrasse 36/2/29  
A-1110 Vienna(AT)  
Inventor: Emmer, Gerhard  
Thereslengasse 25  
A-1180 Vienna(AT)

(54) Heteroatoms-containing tricyclic compounds.

(57) The invention concerns the compounds of formula I

**EP 0 427 680 A1**



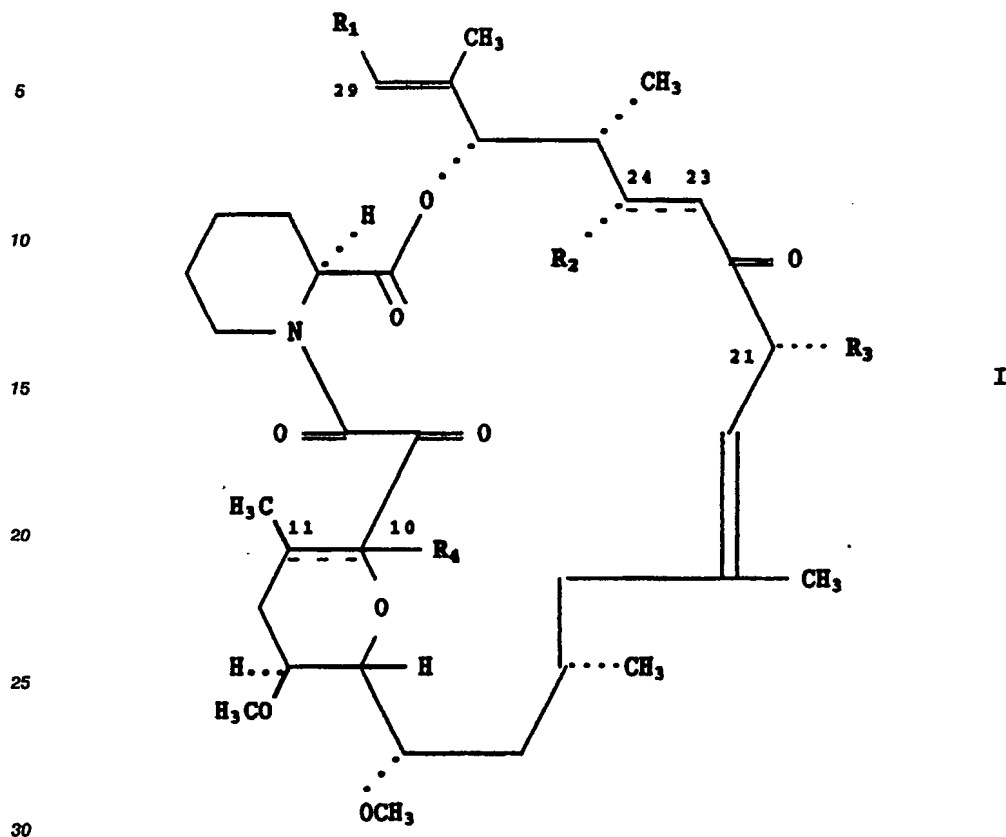
wherein the substituents have various significances.

They are prepared by several processes including epimerizing replacement, treatment with cyanogen bromide or thiophosgene, treatment with an acid having a non-nucleophilic anion, treatment with dimethylsulfoxide and acetic anhydride, acylation, treatment with an oxalyl derivative and ammonia, methylation, oxidation, deprotection and protection.

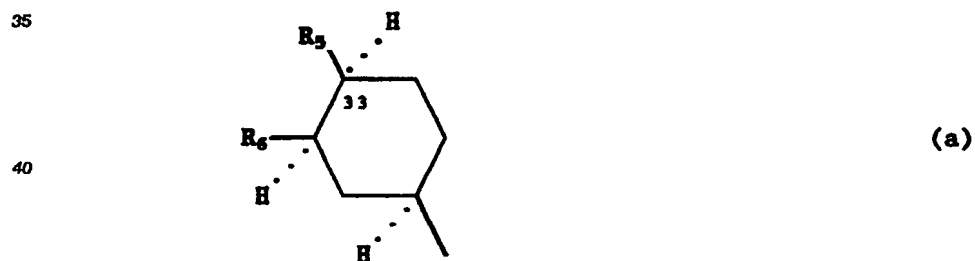
They possess interesting pharmacological activity as antiinflammatory, immunosuppressant, antiproliferative and chemotherapeutic drug resistance reversing agents.

## HETEROATOMS-CONTAINING TRICYCLIC COMPOUNDS

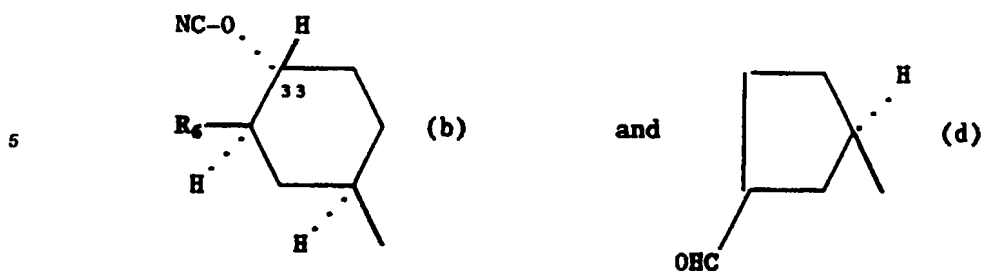
The invention relates to the field of macrolides. It concerns the compounds of formula I



wherein  
either R<sub>1</sub> is a group (a) of formula

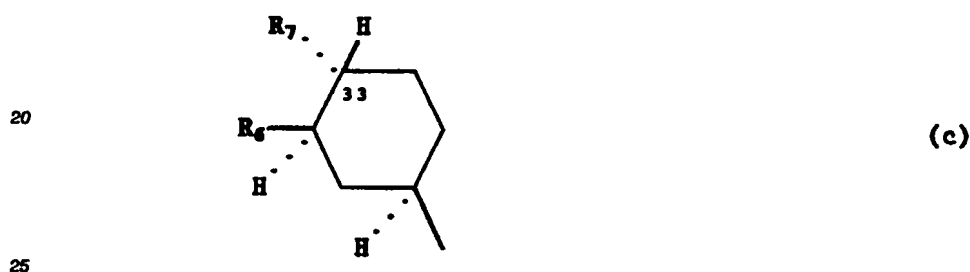


45 wherein R<sub>5</sub> is chloro, bromo, iodo or azido and  
R<sub>6</sub> is hydroxy or methoxy;  
R<sub>2</sub> is oxo and there is a single bond in 23,24 position; optionally protected hydroxy and there is a single or  
a double bond in 23,24 position; or absent and there is a double bond in 23,24 position; and  
50 R<sub>4</sub> is hydroxy and there is a single bond in 10,11 position; or absent and there is a double bond in 10,11  
position;  
or R<sub>1</sub> is a group (b) or (d) of formula



wherein  $R_6$  is as defined above;  
 $R_2$  is as defined above; and  
 $R_4$  is hydroxy and there is a single bond in 10,11 position;  
 or  $R_1$  is a group (c) of formula

15



wherein  $R_6$  is as defined above and

$R_7$  is oxo; optionally protected hydroxy; methoxy; methylthiomethoxy; isobutanoyloxy; aminooxalyloxy;  
 $R_8R_9CHCOO^-$  wherein  $R_8$  is optionally protected hydroxy or optionally protected amino and  $R_9$  is hydrogen  
 or methyl; or p-tolyloxythiocarbonyloxy;

30

$R_2$  is oxo and there is a single bond in 23,24 position; absent and there is a double bond in 23,24 position;  
 or is optionally protected hydroxy, methoxy, methylthiomethoxy, isobutanoyloxy, aminooxalyloxy or  $R_8R_9CH$   
 $COO^-$  wherein  $R_8$  and  $R_9$  are as defined above, and there is a single or a double bond in 23,24 position;  
 whereby for group (c)

- 35
- 1) when  $R_7$  is oxo, unprotected hydroxy or methoxy  
 then  $R_2$  is other than absent and other than unprotected hydroxy or methoxy, and  
 there is a single bond in 23,24 position;
  - 2) when  $R_6$  is methoxy and  $R_7$  is methylthiomethoxy  
 then  $R_2$  is other than absent and other than unprotected hydroxy;
  - 40 3) when  $R_6$  is methoxy and  $R_7$  is protected hydroxy  
 then  $R_2$  is other than optionally protected hydroxy; and
  - 4) when  $R_6$  is hydroxy  
 then  $R_7$  is other than optionally protected hydroxy; and  
 $R_4$  is hydroxy and there is a single bond in 10,11 position; and
  - 45  $R_3$  is methyl, ethyl, n-propyl or allyl;  
 in free form and, where such forms exist, in salt form,  
 hereinafter referred to as "the compounds of the invention".

As is evident from formula I and the definition of the substituents when there is a single bond in 10,11  
 position the carbon atom to which the methyl group in 11 position is attached has the  $\beta$ -configuration and  
 there is a hydrogen atom with the  $\alpha$ -configuration attached to the carbon atom in 11 position; when there is  
 a double bond in 10,11 position this methyl group lies in the plane of the paper and there is no hydrogen  
 atom in 11 position. When  $R_2$  is oxo no hydrogen atom is attached to the carbon atom in 24 position. When  
 $R_7$  is oxo the hydrogen atom shown in group (c) attached to the same carbon atom as  $R_7$  is absent.

50

$R_1$  preferably is a group (c) or (d).  $R_2$  preferably is unprotected hydroxy and there is a single bond in  
 23,24 position.  $R_3$  preferably is ethyl or allyl.  $R_4$  preferably is hydroxy.  $R_5$  preferably is chloro.  $R_6$   
 preferably is methoxy.  $R_7$  preferably is isobutanoyloxy, aminooxalyloxy or  $R_8R_9CHCOO^-$ .  $R_8$  preferably is  
 unprotected hydroxy or unprotected amino, especially unprotected hydroxy.  $R_9$  preferably is hydrogen.  
 When  $R_9$  is other than hydrogen the carbon atom to which it is attached preferably has the (S)

configuration.

Protected hydroxy preferably is hydroxy protected by a conventional hydroxy-protecting group such as formyl, tert-butoxycarbonyl, or trialkylsilyl; it especially is tert-butyldimethylsilyloxy.

Optionally protected hydroxy as defined above under formula I for  $R_2$  and  $R_7$  should not be understood as including a group  $R_2$  or  $R_7$  which is otherwise specified, such as e.g. aminooxalyloxy or  $R_8R_9CHCOO^-$ .

Protected amino preferably is amino protected by a conventional amino-protecting group such as benzyloxycarbonyl or trialkylsilyl; it especially is tert-butoxycarbonyl.

A compound of the invention preferably is in free form. It preferably is in unprotected form.

A subgroup of compounds of the invention is the compounds  $Ip_1$ , i.e. the compounds of formula I wherein

$R_1$  is a group (a) wherein  $R_6$  is methoxy and

either  $R_5$  is chloro or bromo and

$R_4$  is hydroxy and there is a single bond in 10,11 position

or  $R_5$  is azido and

$R_4$  is hydroxy and there is a single bond in 10,11 position or absent and there is a double bond in 10,11 position;

$R_2$  is optionally protected hydroxy and there is a single or a double bond in 23,24 position; and

$R_3$  is as defined above under formula I;

in free form and, where such forms exist, in salt form.

A further subgroup of compounds of the invention is the compounds  $Ip_2$ , i.e. the compounds of formula I wherein

$R_1$  is a group (c) wherein  $R_6$  is methoxy and  $R_7$  is oxo; optionally protected hydroxy; methoxy; methylthiomethoxy; aminooxalyloxy;  $R_8CH_2COO^-$  wherein  $R_8$  is optionally protected amino; or p-tolylox-ythiocarbonyloxy;

$R_2$  is absent and there is a double bond in 23,24 position; or optionally protected hydroxy, methoxy, methylthiomethoxy or aminooxalyloxy and there is a single or double bond in 23,24 position;

whereby

1) when  $R_7$  is oxo, unprotected hydroxy or methoxy

then  $R_2$  is other than absent and other than unprotected hydroxy or methoxy, and

there is a single bond in 23,24 position;

2) when  $R_7$  is methylthiomethoxy

then  $R_2$  is other than absent and other than unprotected hydroxy; and

3) when  $R_7$  is protected hydroxy

then  $R_2$  is other than optionally protected hydroxy; and

$R_4$  is hydroxy and there is a single bond in 10,11 position; and

$R_3$  is as defined above under formula I;

in free form and, where such forms exist, in salt form.

A further subgroup of compounds of the invention is the compounds  $Ip_3$ , i.e. the compounds of formula I wherein

$R_1$  is a group (b) wherein  $R_6$  is methoxy,

$R_2$  is optionally protected hydroxy and there is a single bond in 23,24 position; or absent and there is a double bond in 23,24 position;

$R_4$  is hydroxy and there is a single bond in 10,11 position; and

$R_3$  is as defined above under formula I;

in free form and, where such forms exist, in salt form.

A further subgroup of compounds of the invention is the compounds  $Ip_4$ , i.e. the compounds of formula I wherein

$R_1$  is a group (d),

$R_2$  is optionally protected hydroxy and there is a single bond in 23,24 position; or absent and there is a double bond in 23,24 position;

$R_4$  is hydroxy and there is a single bond in 10,11 position; and

$R_3$  is as defined above under formula I;

in free form and, where such forms exist, in salt form.

A preferred subgroup of compounds of the invention is the compounds of formula I wherein

$R_1$  is a group (a) wherein  $R_5$  is as defined above under formula I and  $R_6$  is methoxy;

$R_2$  is optionally protected hydroxy and there is a single bond in 23,24 position;

$R_4$  is hydroxy and there is a single bond in 23,24 position; or absent and there is a double bond in 10,11 position; and

R<sub>3</sub> is ethyl or allyl.

A further preferred group of compounds of the invention is the compounds of formula I wherein

R<sub>1</sub> is a group (b) wherein R<sub>6</sub> is methoxy;

R<sub>2</sub> is optionally protected hydroxy and there is a single bond in 23,24 position; or absent and there is a double bond in 23,24 position;

R<sub>4</sub> is hydroxy and there is a single bond in 10,11 position; and

R<sub>3</sub> is ethyl or allyl.

A further preferred group of compounds of the invention is the compounds of formula I wherein

R<sub>1</sub> is a group (c) wherein R<sub>6</sub> is methoxy and R<sub>7</sub> is as defined above under formula I;

R<sub>2</sub> is oxo and there is a single bond in 23,24 position; or optionally protected hydroxy, methylthiomethoxy, aminooxalyloxy, R<sub>8</sub>CH<sub>2</sub>COO- wherein R<sub>8</sub> is optionally protected amino, and there is a single or a double bond in 23,24 position;

whereby

1) when R<sub>7</sub> is oxo, unprotected hydroxy or methoxy

then R<sub>2</sub> is other than unprotected hydroxy or methoxy, and there is a single bond in 23,24 position;

2) when R<sub>7</sub> is methylthiomethoxy

then R<sub>2</sub> is other than unprotected hydroxy; and

3) when R<sub>7</sub> is protected hydroxy

then R<sub>2</sub> is other than optionally protected hydroxy;

R<sub>4</sub> is hydroxy and there is a single bond in 10,11 position; and

R<sub>3</sub> is ethyl or allyl.

A further preferred subgroup of compounds of the invention is the compounds of formula I wherein

R<sub>1</sub> is a group (d),

R<sub>2</sub> is optionally protected hydroxy and there is a single bond in 23,24 position; or absent and there is a double bond in 23,24 position;

R<sub>4</sub> is hydroxy and there is a single bond in 10,11 position; and

R<sub>3</sub> is ethyl or allyl.

A further subgroup of compounds of the invention is the compounds Iq, i.e. the compounds of formula I wherein

either R<sub>1</sub> is a group (a) wherein R<sub>5</sub> is chloro, bromo, iodo or azido and R<sub>6</sub> is hydroxy or methoxy,

R<sub>2</sub> is oxo and there is a single bond in 23,24 position; optionally protected hydroxy and there is a single or a double bond in 23,24 position; or absent and there is a double bond in 23,24 position; and

R<sub>4</sub> is hydroxy and there is a single bond in 10,11 position; or absent and there is a double bond in 10,11 position;

or R<sub>1</sub> is a group (b) or (d) wherein R<sub>6</sub> is hydroxy or methoxy;

R<sub>2</sub> is as defined above for this subgroup; and

R<sub>4</sub> is hydroxy and there is a single bond in 10,11 position;

or R<sub>1</sub> is a group (c) wherein

R<sub>6</sub> is hydroxy or methoxy and

R<sub>7</sub> is aminooxalyloxy; R<sub>8</sub>R<sub>9</sub>CHCOO- wherein R<sub>8</sub> is optionally protected hydroxy or optionally protected amino and R<sub>9</sub> is hydrogen or methyl; or p-tolyloxythiocarbonyloxy;

R<sub>2</sub> is methylthiomethoxy, isobutanoyloxy, aminooxalyloxy or R<sub>8</sub>R<sub>9</sub>CHCOO- wherein R<sub>8</sub> and R<sub>9</sub> are as defined above for this subgroup, and there is a single or double bond in 23,24 position; and

R<sub>4</sub> is hydroxy and there is a single bond in 10,11 position; and

R<sub>3</sub> is methyl, ethyl, n-propyl or allyl,

in free form and, where such forms exist, in salt form.

A further subgroup of compounds of the invention is the compounds Ir, i.e. the compounds of formula I wherein

either R<sub>1</sub> is a group (a) as defined above under formula I; and

R<sub>2</sub> and R<sub>4</sub> have the significance indicated above under group (a);

or R<sub>1</sub> is a group (b) or (d) as defined above under formula I; and

R<sub>2</sub> and R<sub>4</sub> have the significance indicated above under groups (b) and (d);

or R<sub>1</sub> is a group (c) as defined above under formula I wherein

R<sub>6</sub> is as defined above under formula I and

R<sub>7</sub> with the exception of optionally protected hydroxy has the significance indicated above under group (c); whereby for group (c)

1) when R<sub>7</sub> is oxo or methoxy

then R<sub>2</sub> is other than absent and other than methoxy, and there is a single bond in 23,24 position; and

2) when R<sub>6</sub> is methoxy and R<sub>7</sub> is methylthiomethoxy

then  $R_2$  is other than absent; and

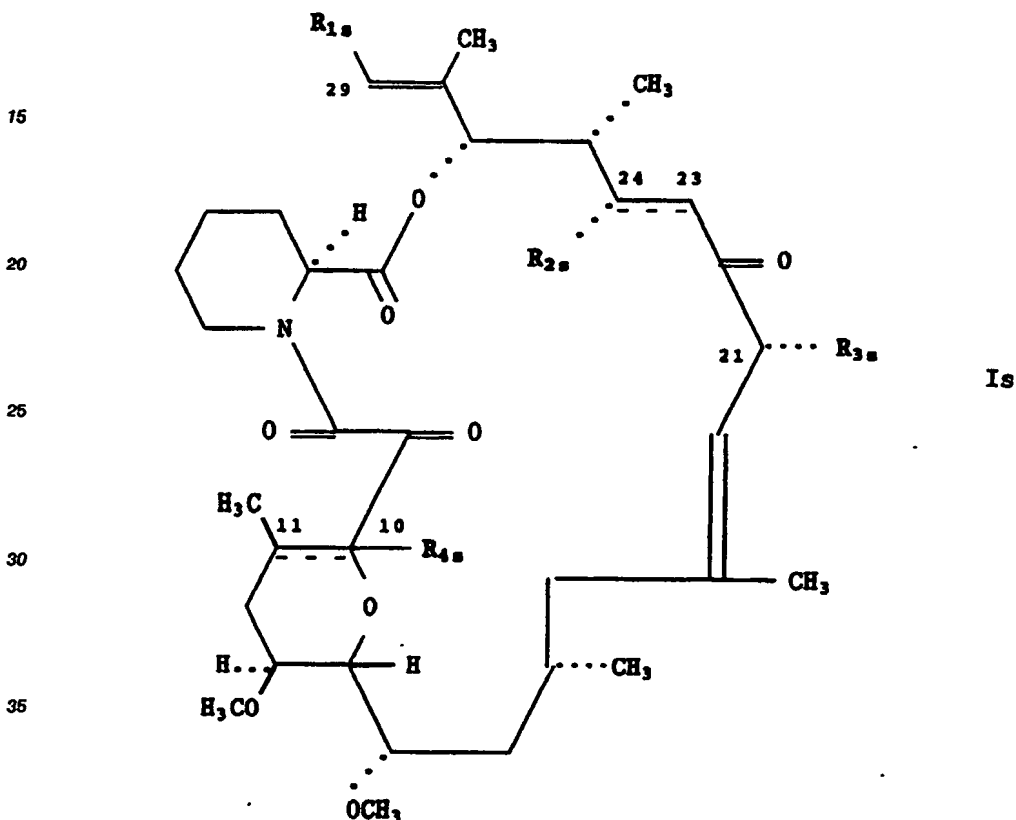
$R_4$  has the significance indicated above under group (c); and

5  $R_3$  is as defined above under formula I;

in free form and, where such forms exist, in salt form.

In a subgroup of compounds  $R_7$  is other than oxo or methoxy; in a further subgroup when  $R_6$  is methoxy then  $R_7$  is other than methylthiomethoxy; in a further subgroup  $R_2$  is other than absent and other than methoxy.

10 A further subgroup of compounds of the invention is the compounds of formula Ia



**wherein**

either R<sub>16</sub> is a group (a) wherein R<sub>5</sub> is chloro, bromo, iodo or azido and R<sub>6</sub> is methoxy;

**R<sub>28</sub>** is hydroxy optionally protected by tert-butyldimethylsilyloxy and there is a single bond in 23,24 position; and

<sup>45</sup> R<sub>4s</sub> is hydroxy and there is a single bond in 10,11 position; or absent and there is a double bond in 10,11 position;

or R<sub>18</sub> is a group (b) wherein R<sub>6</sub> is methoxy, or a group (d);

**R<sub>2a</sub>** is hydroxy optionally protected by tert-butyldimethylsilyloxy and there is a single bond in 23,24 position; or absent and there is a double bond in 23,24 position; and

<sup>50</sup> R<sub>4a</sub> is hydroxy and there is a single bond in 10,11 position;

or  $R_{1s}$  is a group (c) wherein

R<sub>6</sub> is methoxy and

R<sub>7</sub> is oxo; hydroxy optionally protected by tert-butyldimethylsilyloxy; methoxy; methylthiomethoxy; isobutanoyloxy; aminooxaloyloxy; R<sub>8</sub>R<sub>9</sub>CHCOO- wherein R<sub>8</sub> is hydroxy optionally protected by tert-butyldimethylsilyloxy or amino optionally protected by tert-butoxycarbonyl and R<sub>9</sub> is hydrogen or methyl; or p-tolylsulfonylthiocarbonyloxy;

**R<sub>23</sub>** is oxo and there is a single bond in 23,24 position; absent and there is a double bond in 23,24 position; or is hydroxy optionally protected by tert-butyldimethylsilyloxy, methoxy, methylthiomethoxy, aminoox-

alyloxy or  $R_8R_9CHCOO-$  wherein  $R_8$  is amino optionally protected by tert-butoxycarbonyl and  $R_9$  is hydrogen, and there is a single bond in 23,24 position;

whereby for group (c)

- 1) when  $R_7$  is oxo, unprotected hydroxy or methoxy  
 5 then  $R_{25}$  is other than absent and other than unprotected hydroxy or methoxy, and there is a single bond in 23,24 position;
- 2) when  $R_7$  is methylthiomethoxy  
 then  $R_{25}$  is other than absent and other than unprotected hydroxy;  
 and
- 10 3) when  $R_7$  is hydroxy protected by tert-butyldimethylsilyloxy  
 then  $R_{25}$  is other than hydroxy optionally protected by tert-butyldimethylsilyloxy; and  
 $R_{45}$  is hydroxy and there is a single bond in 10,11 position; and  
 $R_{35}$  is ethyl or allyl,  
 in free form and, where such forms exist, in salt form.

15 A compound of the invention can be obtained by a process comprising

a) for the preparation of a compound of formula I wherein

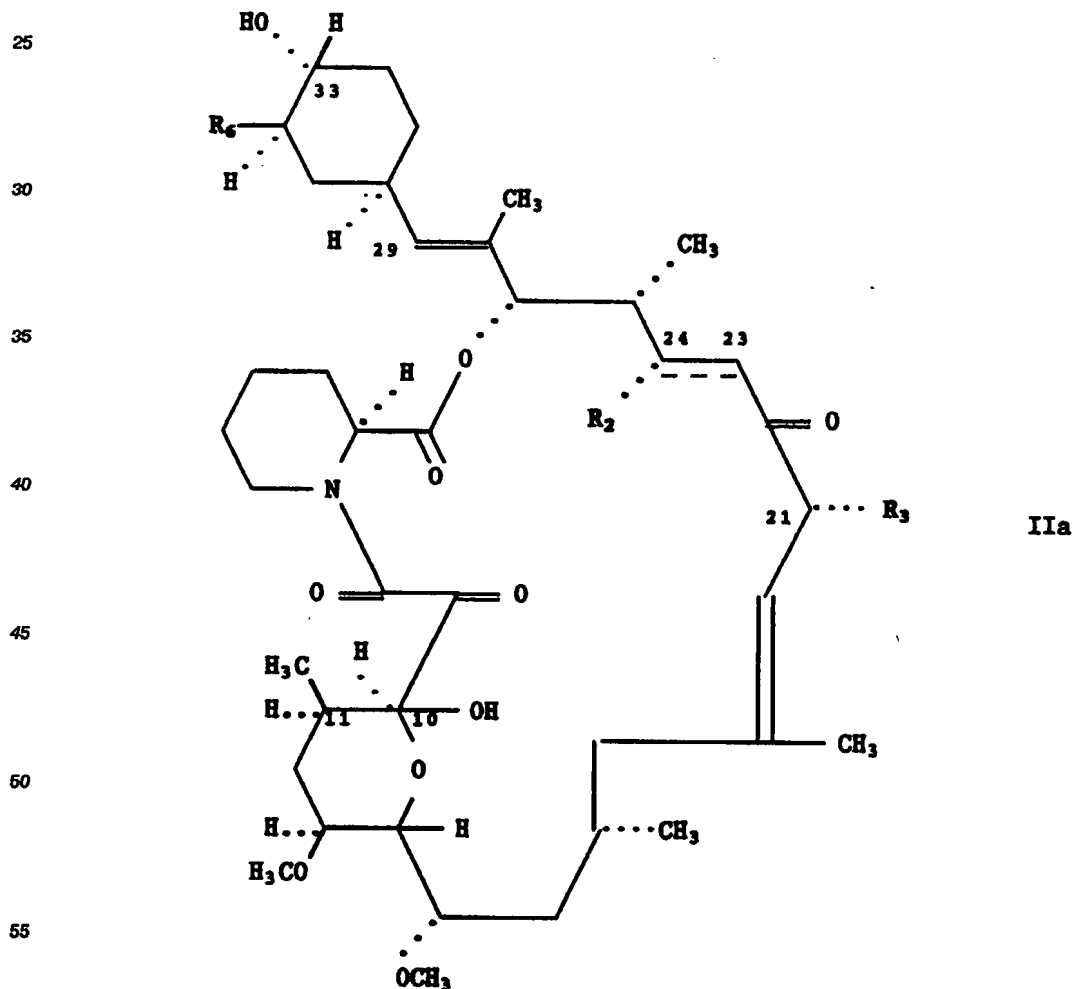
$R_1$  is a group (a) as defined above under formula I,

$R_2$  and  $R_3$  are as defined above under formula I and

$R_4$  is hydroxy

20 (i.e. a compound Ia),

replacing under simultaneous epimerization the hydroxy group by chlorine, bromine, iodine or azido in a corresponding compound having unprotected hydroxy in 33 position (i.e. a compound IIa, of formula IIa





wherein  $R_2$  and  $R_3$  are as defined above under formula I and  $R_6$  is hydroxy or methoxy);

b) for the preparation of a compound of formula I wherein

$R_1$  is a group (b) as defined above under formula I,

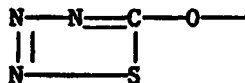
5  $R_2$  and  $R_3$  are as defined above under formula I and

$R_4$  is hydroxy

(i.e. a compound Ib),

treating a corresponding compound IIa with cyanogen bromide in the presence of a base or

10 treating a corresponding compound IIa with thiophosgene, reacting the resultant product with an inorganic azide and allowing the resultant unstable intermediate having a group



15

in 33 position (i.e. a compound IIb)

to decompose to a corresponding compound Ib;

c) for the preparation of a compound of formula I wherein

20  $R_1$  is a group (d) as defined above under formula I,

$R_2$  and  $R_3$  are as defined above under formula I and

$R_4$  is hydroxy

(i.e. a compound Ic),

treating a corresponding compound Ib with an acid having a non-nucleophilic anion;

d) for the preparation of a compound of formula I wherein

25  $R_1$  is a group (c) wherein  $R_6$  is as defined above under formula I, one of  $R_2$  and  $R_7$  is oxo or methylthiomethoxy and the other is protected hydroxy,

$R_3$  is as defined above under formula I and

$R_4$  is hydroxy

(i.e. a compound Id),

30 treating a corresponding compound wherein

one of the substituents in 24 and 33 position is hydroxy and the other is protected hydroxy,

(i.e. a compound IIc)

with dimethylsulfoxide and acetaldehyde;

e) for the preparation of a compound of formula I wherein

35  $R_1$  is a group (c) wherein

$R_6$  is as defined above under formula I and

$R_7$  is isobutanoyloxy, aminooxalyloxy,  $R_8R_9CHCOO^-$  as defined above under formula I or p-tolyloxythiocarbonyloxy,

40  $R_2$  and  $R_3$  are as defined above under formula I and

$R_4$  is hydroxy

(i.e. a compound Ie),

appropriately acylating a corresponding compound IIa;

f) for the preparation of a compound of formula I wherein

45  $R_1$  is a group (c) wherein

$R_6$  is as defined above under formula I and

$R_7$  is aminooxalyloxy,

$R_2$  is optionally protected hydroxy or is aminooxalyloxy,

$R_3$  is as defined above under formula I and

$R_4$  is hydroxy

50 (i.e. a compound If),

treating with a appropriate oxalyl derivative and thereafter with ammonia a corresponding compound having a optionally protected hydroxy group in 33 position and a protected hydroxy group in 24 position

(i.e. a compound IId);

g) for the preparation of a compound of formula I wherein

55  $R_1$  is a group (c) wherein  $R_6$  is as defined above under formula I,

$R_2$  and  $R_7$  are as defined above under formula I with the proviso that one of  $R_2$  and  $R_7$  is methoxy,

$R_3$  is as defined above under formula I and

R<sub>4</sub> is hydroxy

(i.e. a **compound Ig**),

methyating a corresponding compound having a hydroxy group in 24 or 33 position

(i.e. a **compound Ile**);

5 h) for the preparation of a compound of formula I wherein

R<sub>1</sub> is a group (c) wherein R<sub>6</sub> is as defined above under formula I,

R<sub>2</sub> and R<sub>7</sub> are as defined above under formula I with the proviso that one of R<sub>2</sub> and R<sub>7</sub> is oxo,

R<sub>3</sub> is as defined above under formula I and

R<sub>4</sub> is hydroxy

10 (i.e. a **compound Ih**),

oxidizing a corresponding compound having a hydroxy group in 24 or 33 position

(i.e. a **compound If**); and

- when a resultant compound of formula I has a protected hydroxy and/or a protected amino group,

optionally splitting off the protecting group(s) to give a corresponding compound of formula I having one

15 or more unprotected hydroxy and/or unprotected amino group(s)

(i.e. a **compound Ij**),

- whereby when R<sub>1</sub> is a group (a), a water molecule may be simultaneously split off and a compound of formula I is obtained wherein

R<sub>1</sub> is a group (a) as defined above under formula I,

20 R<sub>2</sub> is unprotected hydroxy and there is a single or double bond in 23,24 position; and

R<sub>4</sub> is absent and there is a double bond in 10,11 position (i.e. a **compound II**); or

- optionally protecting an unprotected hydroxy and/or unprotected amino group in a resultant compound of formula I as appropriate to give a corresponding compound of formula I having one or more protected hydroxy and/or protected amino groups(s) (i.e. a **compound Ik**),

25 and recovering the resultant compound of formula I in free form and, where such forms exist, in salt form.

The process variants of the invention can be effected in a manner analogous to known procedures.

Process variant a) is a substitution reaction under simultaneous epimerization. It is preferably effected in an inert solvent such as tetrahydrofuran or toluene. Preferably for the substitution by halogen the reaction 30 is effected with tetrachloro-, tetrabromo- or tetraiodomethane in the presence of triphenylphosphine, and for the substitution by azido with azodicarboxylic acid ester, preferably diethyl ester, and hydrazoic acid. A hydroxy group in 24 position may be in protected form. As protecting group known hydroxy protecting groups such as tert-butyldimethylsilyl may be used. A protecting group may subsequently be split off in accordance with known procedures, e.g. with hydrofluoric acid in acetonitrile. Upon deprotection a water 35 molecule may, depending on the reaction conditions chosen, simultaneously be split off in position 10,11 and a double bond formed. The individual compounds can be separated from such a resultant mixture in conventional manner, e.g. chromatographically.

Compounds Ia may be further processed by e.g. oxidation or dehydration to corresponding compounds wherein R<sub>4</sub> is absent; for example, oxidation of compounds Ia wherein R<sub>2</sub> is hydroxy leads to corresponding 40 compounds wherein R<sub>4</sub> is absent and R<sub>2</sub> is oxo.

Process variant b) is a cyanidation reaction. It preferably is effected in an inert solvent such as a chlorinated hydrocarbon, e.g. dichloromethane. The temperature preferably is about room temperature. The base is e.g. 4-dimethylaminopyridine.

A compound of formula I obtained accordance to process variants a) and b) above may be isolated 45 from the reaction mixture and purified in accordance with known methods. When R<sub>2</sub> is hydroxy and there is a single bond in 23,24 position a water molecule may be simultaneously split off. A corresponding mixture of compounds Ib is obtained wherein either R<sub>2</sub> is hydroxy and there is a single bond in 23,24 position or R<sub>2</sub> is absent and there is a double bond in 23,24 position. The individual compounds can be separated from such a resultant mixture in conventional manner, e.g. chromatographically.

50 The second procedure according to process variant b) is effected by reaction with thiophosgene, preferably in the presence of an acid scavenger such as 4-dimethylaminopyridine. Preferably an inert solvent such as acetonitrile is used. The temperature preferably is about room temperature. The subsequent reaction with an inorganic azide is preferably effected with sodium azide. The resultant compounds IIb are unstable and decompose already at room temperature to compounds Ib, under splitting off of nitrogen 65 and sulfur. This reaction step preferably is effected in an inert solvent such as an aromatic hydrocarbon, e.g. benzene. Temperature preferably is elevated, e.g. about 50 °C.

In process variant c) a ring contraction takes place. Protecting groups which are present may be simultaneously split off. Preferably an inert solvent such as acetonitrile is used. Preferably hydrofluoric acid

is used as acid having a non-nucleophilic anion. Temperature preferably is about room temperature.

Process variant d) is a Swern oxidation. The reaction preferably is effected with compound IIc dissolved in dimethylsulfoxide and acetic anhydride. Duration of reaction is prolonged, e.g. about 5 hours. Temperature preferably is about room temperature.

5 Process variant e) is an acylation. It is preferably effected in an inert solvent such as acetonitrile. The acylating agent preferably is an activated acyl derivative, such as a acyl halogenide or anhydride. An acid scavenger such as dimethylaminopyridine or pyridine is employed. Further, a compound IIa may also be reacted with a carboxylic acid such as glycine protected at the amino moiety by e.g. tert-butoxycarbonyl, or with a compound of formula  $R_8R_9CHCOOH$  wherein  $R_8$  is protected hydroxy and  $R_9$  is hydrogen or methyl, and a carbodiimide such as N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide or N,N'-dicyclo-hexylcarbodiimide, where indicated in the presence of a base, such as 4-dimethylaminopyridine, preferably in an inert solvent such as acetonitrile or in a chlorinated hydrocarbon. An amino protecting group may subsequently be split off together with any hydroxy protecting group which may be present. If in the starting compound IIa  $R_2$  is hydroxy and there is a single bond in 23,24 position, upon acylation splitting off of a water molecule in 23,24 position may occur and a compound IIe be formed wherein  $R_2$  is absent and there is a double bond in 23,24 position.

Process variant f) is an acylation. It is preferably effected in an inert solvent such as acetonitrile. Temperature preferably is reduced, e.g. about 0 to 5 °C. The oxalyl derivative preferably is an oxalyl halogenide, e.g. chloride. Upon completion of the reaction the mixture is stirred with ammonia.

20 Process variant g) is a methylation. It preferably is effected in an inert solvent such as a chlorinated hydrocarbon, e.g. dichloromethane. The methylating agent preferably is diazomethane in the presence of e.g. borotrifluoride-etherate. Temperature preferably is from about 0 ° to about room temperature.

Process variant h) is an oxidation. The oxidizing agent is e.g. tetrapropylammonium perruthenate. The temperature preferably is about room temperature.

25 The optional deprotection process variant may also be effect in conventional manner. For splitting off of e.g. tert-butyldimethylsilyl it is effected by treatment with e.g. hydrofluoric acid in a solvent such as acetonitrile. Depending on the reaction conditions selected (duration, temperature, etc.) the splitting can be steered in such a manner that either all or only some protecting group are removed. Partial deprotection is particularly indicated where a definite hydroxy group is to be subsequently reacted in a later reaction.

30 The optional protection step variant may also be effected in conventional manner along similar lines.

Thus for subsequent reactions involving a hydroxy group, particularly a hydroxy group in position 24 and/or 33, selective protection of only one of the two free hydroxy groups or selective deprotection of only one of the two protected hydroxy groups may be effected in such a manner that reaction occurs only at the desired position. Mixtures of end products may be obtained thereby; such mixtures can be separated in conventional manner, e.g. chromatographically. Resultant end products still containing protecting groups can be subsequently deprotected. Reaction conditions may alternatively be selected such that simultaneously with or immediately after reaction the protecting groups are removed (one pot process).

The compounds of formula I may be isolated and purified from the reaction mixture in conventional manner.

40 Insofar as their preparation is not specifically described herein, e.g. in the Examples; the compounds used as starting materials are known or can be obtained in conventional manner from known compounds, e.g. starting from appropriate Streptomyces strains such as Streptomyces tsukubaensis No. 9993 described in e.g. Fujisawa EP 184162. Samples can be obtained from the Fermentation Research Institute, Tsukuba, Ibaraki 305, Japan under provisions of the Budapest Treaty under deposit No. FERM BP-927. This strain has been redeposited on April 27, 1989 e.g. as disclosed in Sandoz EP 356399, with the Agricultural Research Culture Collection International Depository, Peoria, Illinois 61604, USA under the provisions of the Budapest Treaty under deposit No. NRRL 18488.

The following Examples illustrate the invention and are not limitative. All temperatures are in degrees Centigrade. All NMR spectra are in  $CDCl_3$ , ppm. The abbreviations have the following meanings:

50 BOC: tert-butoxycarbonyl;

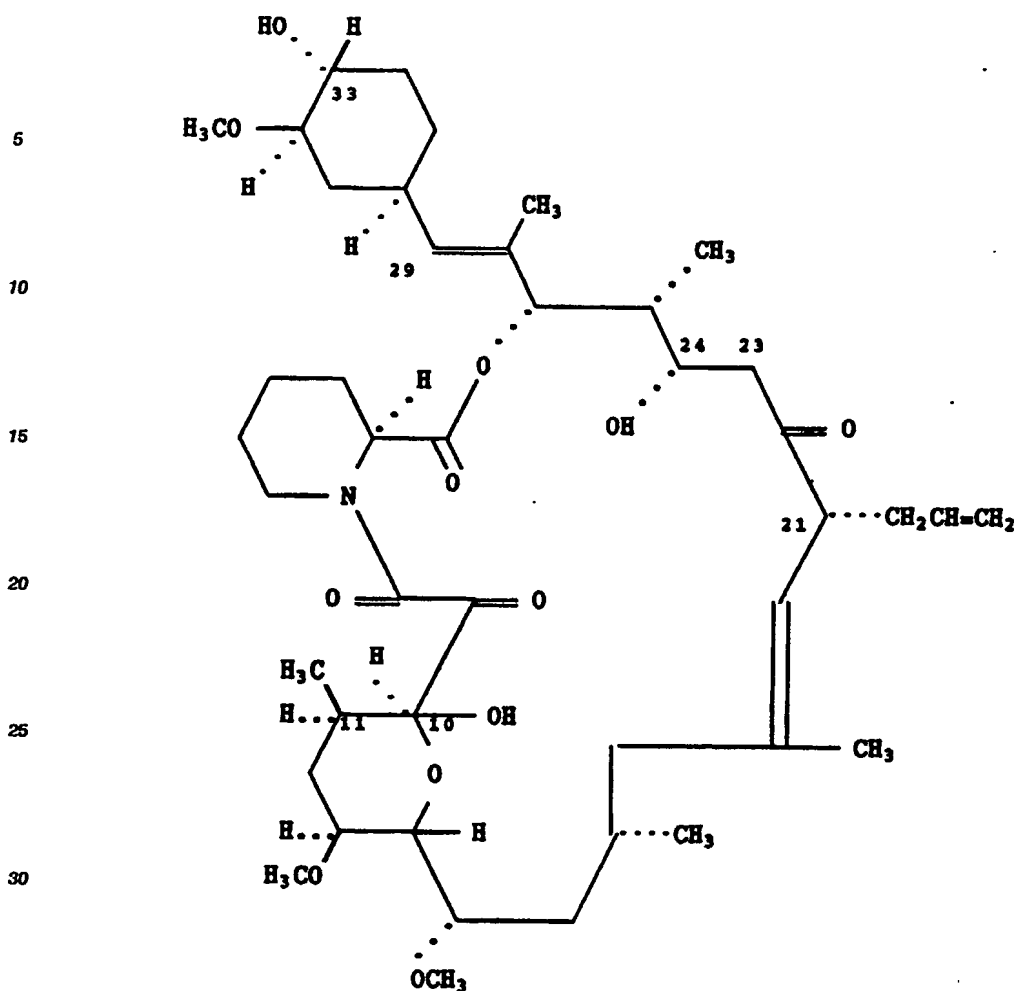
cfr: colourless foamy resin;

db: double bond;

Et: ethyl;

FK 506: the compound of formula

55



i.e. 17 $\alpha$ -allyl-1 $\beta$ ,14 $\alpha$ -dihydroxy-12-[2'-(4''(R)-hydroxy-3''(R)-methoxycyclohex-1''(R)-yl)-1'-methyl-trans-vinyl]-23 $\alpha$ ,25 $\beta$ -dimethoxy-13 $\alpha$ ,19,21 $\alpha$ ,27 $\beta$ -tetramethyl-11,28-dioxo-4-azatricyclo[22.3.1.0<sup>4,5</sup>]octacos-18-trans-ene-2,3,10,16-tetraone (according to the atom numbering in EP 184162; however, in the Examples the atom numbering of formula I is used throughout);

FR 520: as FK 506, but with  $\text{---CH}_2\text{CH}_3$  (ethyl) in place of allyl in position 21 in the formula;

iBuoyloxy: isobutanoyloxy [(H<sub>3</sub>C)<sub>2</sub>CHCOO-];

iPr: isopropyl;

na: not applicable;

N<sub>3</sub>: azido;

OMe (or MeO): methoxy;

OtBDMS: tert-butyldimethylsilyloxy;

sb: single bond;

tBu: tert-butyl.

#### Example 1: 24-tert-Butyldimethylsilyloxy-33-epi-33-chloro-FK506

[Formula 1: R<sub>1</sub> = a group (a) wherein R<sub>5</sub> = chloro, R<sub>6</sub> = OMe; R<sub>2</sub> = OtBDMS, single bond in 23,24 position; R<sub>3</sub> = allyl; R<sub>4</sub> = OH, single bond in 10,11 position]

[Process variant a), replacement with epimerization]

0.092 g 24-*tert*-butyldimethylsilyloxy-FK506 is heated for 15 hours under refluxing with 0.037 g triphenylphosphine in 4 ml of tetrachloromethane. The solvent is evaporated to dryness under reduced pressure and the residue is purified by column chromatography over silicagel using a mixture of hexane and acetic acid ethyl ester (2:1) as the eluant. The title compound is obtained (colourless foam):

5 <sup>1</sup>H-NMR: about 2:3 mixture of conformers:

main conformer: 4.56 (m,  $w_{1/2}$  = 7 Hz, H-33).

The starting material is obtained as follows:

a) 20 g FK 506 is dissolved in 400 ml of dry dimethylformamide, 5.08 g imidazole and 11.25 g *tert*-butyldimethylchlorosilane is added in portions and the mixture is stirred for 110 hours at room temperature. The reaction mixture is diluted with acetic acid ethyl ester and washed five times with water. The organic phase is dried over sodium sulfate and the solvent distilled off under reduced pressure. The resultant crude product is purified by chromatography over silicagel using hexane/acetic acid ethyl ester 3:1 as the eluant. 24,33-Bis-(*tert*-butyldimethylsilyloxy)-FK 506 is obtained:

10 <sup>13</sup>C-NMR: main conformer: 69.7 (C-24); 75.1 (C-33); 84.1 (C-32); 164.6 (C-8); 168.9 (C-1); 196.4 (C-9); 209.3 (C-22);

15 minor conformer: 70.9 (C-24); 75.3 (C-33); 84.1 (C-32); 165.8 (C-8); 168.2 (C-1); 191.2 (C-9); 210.0 (C-22);

b) 0.5 g 24,33-bis-(*tert*-butyldimethylsilyloxy)-FK506 is dissolved at 0° under stirring into a mixture of 10 ml of acetonitrile and 0.5 ml of 40 % hydrofluoric acid. After 2 hours at that temperature the reaction medium is diluted with dichloromethane. The solution is successively washed with saturated aqueous sodium bicarbonate solution and water and the organic phase is dried over sodium sulfate, and the solvent is evaporated under reduced pressure. The resultant residue is purified by column chromatography over silicagel (eluant: dichloromethane/methanol 9:1). 24-*tert*-Butyldimethylsilyloxy-FK 506 is obtained as a colourless foam:

25 <sup>13</sup>C-NMR: main conformer: 69.7 (C-24); 73.6 (C-33); 84.1 (C-32); 164.6 (C-8); 168.9 (C-1); 196.4 (C-9); 209.2 (C-22);

minor conformer: 70.7 (C-24); 73.6 (C-33); 84.2 (C-32); 165.8 (C-8); 168.2 (C-1); 191.4 (C-9); 209.2 (C-22).

30

#### Example 2: 24-*tert*-Butyldimethylsilyloxy-33-*epi*-33-azido-FK506

[Formula I: R<sub>1</sub> : a group (a) wherein R<sub>5</sub> : azido, R<sub>6</sub> : OMe; R<sub>2</sub> : OtBDMS, single bond in 23,24 position; R<sub>3</sub> = allyl; R<sub>4</sub> = OH, single bond in 10,11 position]

35

[Process variant a)]

40

To a solution of 0.092 g 24-*tert*-butyldimethylsilyloxy-FK506 and 0.08 g triphenylphosphine in 2 ml of dry tetrahydrofuran is added at 0° 0.047 ml of azodicarboxylic acid diethyl ester, followed by 0.15 ml of a 2 M solution of hydrazoic acid in toluene. The solution is brought to room temperature and stirred for 18 hours. The solvent is evaporated to dryness under reduced pressure and the residue purified as described above under Example 1. The title compound is obtained (colourless foam):

45 <sup>1</sup>H-NMR: 4.07 (m, H-33).

50

55

The following compounds of formula I are obtained in analogous manner in accordance with process variant a):

Example No.	Analogous to				R <sub>7</sub>	R <sub>2</sub>	Position 23,24	R <sub>3</sub>	R <sub>4</sub>	Position 10,11	Physicochemical characterization data
	R <sub>1</sub>	R <sub>5</sub>	R <sub>6</sub>	R <sub>7</sub>							
3	1 <sup>1)</sup>	(a)	Cl	OMe	na	OtBDMS	sb	Et	OH	sb	NMR*
4	1 <sup>1)</sup>	(a)	Br	OMe	na	OtBDMS	sb	Et	OH	sb	
5	1	(a)	Br	OMe	na	OtBDMS	sb	allyl	OH	sb	
6	2 <sup>1)</sup>	(a)	N <sub>3</sub>	OMe	na	OtBDMS	sb	Et	OH	sb	
6a	1 <sup>1)</sup>	(a)	I	OMe	na	OtBDMS	sb	Et	OH	sb	NMR*

\*NMR: Example 3: <sup>1</sup>H-NMR: 4.56 (m, H-33);

Example 6a: <sup>13</sup>C-NMR: mixture of conformers: 210.33 (C-22); 168.91 (C-1); 164.59 (C-8); 123.64 (C-20); 78.90 (C-32); 25.81 (tBu);

1) The starting material is obtained from FR 520 in a manner analogous to 24-tert-butylidimethylsilyloxy-FK 506 (see Example 1):

a) 24,33-bis-(tert-butylidimethylsilyloxy)-FR 520:<sup>1</sup>H-NMR: about 2:1 mixture of 2 conformers:  
 main conformer: 4.42 (m, H-2); 4.41 (db, 13 Hz, H-6 eq.); 4.05 (txt, J=1.5 Hz and 6 Hz, H-24); 3.80 (dxd, J=1.5 Hz and 10 Hz, H-14); 2.95 (dxdxd, J=4 Hz, 8 Hz and 11 Hz, H-32);  
 minor conformer: 4.25 (q, J=5 Hz, H-24); 3.94 (dxd, J=2 Hz and 10 Hz, H-14); 2.95 (dxdxd, J=4 Hz, 8 Hz and 11 Hz, H-32);

b) 24-tert-butylidimethylsilyloxy-PB 520: <sup>1</sup>H-NMR: about 2:1 mixture of 2 conformers:  
main conformer: 4.44 (b, H-2); 4.42 (db, J=13 Hz, H-6 eq.);  
4.05 (dxt, J=1,5 Hz and 6 Hz, H-24); 3.81 (dxd, J=1.5 Hz  
and 10 Hz, H-14); 3.01 (dxdxd, J=4 Hz, 8 Hz and 11 Hz, H-32);  
minor conformer: 4.24 (H-24); 3.94 (dxd, J=2 Hz and 10 Hz,  
H-14); 3.01 (dxdxd, J=4 Hz, 8 Hz and 11 Hz, H-32).

---

**Example 7: 24-tert-Butyldimethylsilyloxy-33-cyanoxy-FR 520**

5

[Formula I:  $R_1 = a$  group (b) wherein  $R_6 = OMe$ ;  $R_2 = OtBDMS$ , single bond in 23,24 position;  $R_3 = Et$ ;  $R_4 = OH$ , single bond in 10,11 position]

10

[Process variant b), treatment with cyanogen bromide]

A solution of 2 g 24-tert-butyldimethylsilyloxy-FR 520 and 0.94 g 4-dimethylaminopyridine in 100 ml of dichloromethane is rapidly reacted at room temperature with a solution of 0.4 g cyanogen bromide in 15 ml of dichloromethane and the mixture is stirred at room temperature for 20 minutes. The mixture is filtered over silicagel (eluant: n-hexane/acetic acid ethyl ester) and the solvent is removed from the relevant fraction under reduced pressure. The title compound is obtained as a colourless foamy resin:  
 $^1H$ -NMR: mixture of conformers: 4.3 (m; H-33).

20

**Example 8: 24-tert-Butyldimethylsilyloxy-33-cyanoxy-FR 520**

[Formula I: as for Example 7]

25

[Process variant b), treatment with thiophosgene and sodium azide]

A solution of 2 g 24-tert-butyldimethylsilyloxy-FR 520 and 2 g 4-dimethylaminopyridine in 50 ml of acetonitrile is carefully reacted with 0.4 ml of thiophosgen and the mixture stirred for 3 hours at room temperature. The reaction mixture is poured onto a well-stirred mixture consisting of 150 ml of acetic acid ethyl ester, 40 ml of saturated aqueous sodium chloride solution and 50 ml of 2 N sodium azide solution, vigorous stirring is continued for 5 minutes and the organic phase is separated. The organic phase is then successively washed with water, 1 N hydrochloric acid solution, water, and saturated aqueous sodium chloride solution, dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue is taken up in about 100 ml of benzene and heated at 30-40° for 2 hours. The benzene is removed under reduced pressure and the title compound is recovered from the residue as a colourless foamy resin by column chromatography over silicagel (eluant: n-hexane / acetic acid ethyl ester):  
 $^1H$ -NMR: see Example 7.

40

45

50

55



The following compounds of formula I are obtained in analogous manner in accordance with process variant b):

Example No.	Analogous to Ex. No.	R <sub>1</sub>	R <sub>5</sub>	R <sub>6</sub>	R <sub>7</sub>	R <sub>2</sub>	Position 23,24	R <sub>3</sub>	R <sub>4</sub>	Position 10,11	Physicochemical characterization data
9	7,8	(b)	na	OMe	na	OtBDMS	sb	allyl	OH	sb	NMR*
10a <sup>1)</sup>	7,8	(b)	na	OMe	na	OH	sb	Et	OH	sb	NMR*
10b <sup>1)</sup>	7,8	(b)	na	OMe	na	absent	sb	Et	OH	sb	NMR*
11a <sup>2)</sup>	7,8	(b)	na	OMe	na	OH	sb	allyl	OH	sb	
11b <sup>2)</sup>	7,8	(b)	na	OMe	na	absent	sb	allyl	OH	sb	

\*NMR: Example 9: <sup>1</sup>H-NMR: mixture of conformers: 4.3 (m, H-33);

Example 10a: <sup>1</sup>H-NMR: mixture of conformers: 5.34 (H-26); 4.63 (db, J = 4 Hz, H-2); 4.44 (db, J = 13 Hz, H-6 eq.); 4.30 (dxdxd, J = 5 Hz, 8 Hz and 11 Hz, H-33); 3.01 (tb, J = 13 Hz, H-6ax.);

Example 10b: <sup>1</sup>H-NMR: 6.81 resp. 6.75 (dxd resp. dxd, J = 5 Hz and 15 Hz resp. 7 Hz and 15 Hz, H-24); 6.2 resp. 6.3 (dxd resp. dxd, J = 2 Hz and 15 Hz resp. 1 Hz and 15 Hz, H-23); 5.29 resp. 5.23 (d resp. d, J = 3 Hz resp. 3 Hz, H-26); 4.3 (m, H-3 );

<sup>12)</sup> A mixture of both compounds is obtained; they can be separated chromatographically (eluant:

n-hexane/acetic acid ethyl ester).

#### **Example 12: 29-Des-(4-hydroxy-3-methoxycyclohexyl)-29-(3-formylcyclopentyl)-FR 520**

[Formula I: R<sub>1</sub> = a group (d); R<sub>2</sub> = OH, single bond in 23,24 position; R<sub>3</sub> = Et; R<sub>4</sub> = OH, single bond in 10,11 position]

[Process variant c), treatment with a non-nucleophilic anion]

0.5 g 24-tert-butyldimethylsilyloxy-33-cyanoxy-FK 520 (compound of Examples 7 and 8) or 33-cyanoxy-FR 520 (compound of Example 10a) is dissolved into a mixture of 50 ml of acetonitrile and 2 ml of 40 % wt. aqueous hydrofluoric acid and the mixture is stirred for 2.5 hours at room temperature. The reaction mixture is then distributed between acetic acid ethyl ester and saturated aqueous sodium bicarbonate solution, the aqueous phase is discarded and the organic phase is washed with saturated aqueous sodium chloride solution, dried over sodium sulfate, filtered and concentrated under reduced pressure. The title compound is obtained as a colourless foamy resin from the residue by column chromatography over silicagel (eluant: n-hexane / acetic acid ethyl ester):

<sup>1</sup>H-NMR: mixture of conformers: 9.64 (d, J = 2 Hz, CHO); 2.87 (m, H-32); 2.67 (m, H-30).

The following compounds of formula I are obtained in analogous manner in accordance with process variant c):											
Example No.	Analogous to Ex. No.	R <sub>1</sub>	R <sub>5</sub>	R <sub>6</sub>	R <sub>7</sub>	R <sub>2</sub>	Position 23,24	R <sub>3</sub>	R <sub>4</sub>	Position 10,11	Physicochemical characterization data
13	12 <sup>1)</sup>	(d)	na	na	na	OH	sb	allyl	OH	sb	NMR*
14	12 <sup>2)</sup>	(d)	na	na	na	absent	db	Et	OH	sb	NMR*

\*NMR: Example 13: <sup>1</sup>H-NMR: mixture of conformers: 9.65 (d, J=2 Hz, CHO); 2.86 (m, H-32); 2.15 (dxdxd, J=12.5 Hz and 7.5 Hz and 5 Hz, H-31a); 1.45 (dxt, J=12.5 and 9 Hz, H-31b); 2.67 (m, H-30); Example 14: <sup>1</sup>H-NMR: about 5:3 mixture of conformers: 9.66 (d, J=2 Hz, CHO); 6.83 (dxd, J=15 and 5 Hz) resp. 6.77 (dxd, J=15 and 7.5 Hz) H-24; 6.19 (dxd, J=15 and 1.5 Hz) resp. 6.30 (dxd, J=15 and 1 Hz) H-23;

<sup>1)</sup> Starting from the compound of Example 9 or 11a;

<sup>2)</sup> Starting from the compound of Example 10b.

#### Example 15:

a) 24-tert-Butyldimethylsilyloxy-33-oxo-FK 506

and

b) 24-tert-Butyldimethylsilyloxy-33-methylthiomethoxy FK 506

[Formula I: R<sub>1</sub> = a group (c) wherein R<sub>6</sub> = OMe, R<sub>7</sub> = oxo and, respectively, methylthiomethoxy; R<sub>2</sub> = OtBDMS, single bond in 23,24 position; R<sub>3</sub> = allyl; R<sub>4</sub> = OH, single bond in 10,11 position]

[Process variant d), treatment with dimethylsulfoxide and acetic anhydride]

1 g 24-tert-Butyldimethylsilyloxy-FK 506 is dissolved at room temperature into a mixture of 20 ml of acetic anhydride and 30 ml of dimethylsulfoxide and stirring is effected for 5 hours at room temperature. The reaction mixture is poured onto a mixture of acetic acid ethyl ester and potassium carbonate solution, stirred for 20 minutes, the phases are separated and the organic phase is repeatedly washed with water, dried over sodium sulfate, filtered and concentrated under reduced pressure. Following column chromatographic fractionation of the residue over silicagel (eluant: acetic acid ethyl ester / n-hexane 2:1) the title compounds are obtained as colourless foamy resins:

compound a): <sup>13</sup>C-NMR: about 2:1 mixture of conformers:

209.3/209.9 (C-22); 208.3/208.5 (C-33); 196.4 (C-9); 168.9/168.2 (C-1); 164.6/165.9 (C-8); 138.5/139.4 (C-19); 135.6/136.1 (C-37); 133.4/134.1 (C-28); 131.8/127.6 (C-29); 123.1/122.3 (C-20); 116.5/116.1 (C-38); 97.6/98.9 (C-10); 83.0 (C-32); 69.6/70.6 (C-24);

compound b): <sup>1</sup>H-NMR: about 2:1 mixture of conformers:

4.82/4.79 (AB; J<sub>AB</sub> = 12 Hz; -O-CH<sub>2</sub>-S); 2.19 resp. 2.18 (s resp. s, -SCH<sub>3</sub>);

The following compounds of formula I are obtained in analogous manner in accordance with process variant d):

Example No.	Analogous to Ex. No.	R <sub>1</sub>	R <sub>5</sub>	R <sub>6</sub>	R <sub>7</sub>	R <sub>2</sub>	Position 23,24	R <sub>3</sub>	R <sub>4</sub>	Position 10,11	Physicochemical characterization data
16a	15 <sup>1)</sup>	(c)	na	OMe	OtBDMS	OCH <sub>2</sub> SCH <sub>3</sub>	sb	allyl	OH	sb	cfr; NMR*
16b	15 <sup>1)</sup>	(c)	na	OMe	OtBDMS	oxo	sb	allyl	OH	sb	cfr; NMR*
16c	15 <sup>2)</sup>	(c)	na	OMe	oxo	OtBDMS	sb	Et	OH	sb	cfr
16d	15 <sup>3)</sup>	(c)	na	OMe	OtBDMS	oxo	sb	Et	OH	sb	cfr

<sup>1)</sup> Starting from 33-tert-butyldimethylsilyloxy-FK 506 (compound of Example 16 in EP 184162); eluant: toluene / acetic acid ethyl ester 9:1;

<sup>2)</sup> Starting from 24-tert-butyldimethylsilyloxy-FR 520;

<sup>3)</sup> Starting from 33-tert-butyldimethylsilyloxy-FR 520 (DOS 39 38 754);

\*NMR: Example 16a: <sup>1</sup>H-NMR: about 2:1 mixture of conformers: 4.36 (s, -O-CH<sub>2</sub>-S) and 2.16 (s, -SCH<sub>3</sub>) resp. 4.37 and 4.40 (AB, -O-CH<sub>2</sub>-S) and 2.13 (s, -SCH<sub>3</sub>);

Example 16b: <sup>1</sup>H-NMR: about 1:1 mixture of conformers: 5.29 and 5.59 (s, H-23);

<sup>13</sup>C-NMR: about 1:1 mixture of conformers: 200.7/197.7 (C-22); 195.3/194.9 (C-24); 193.2/189.6 (C-9);

168.9/169.1 (C-1); 164.4/165.2 (C-8); 137.5/137.9 (C-19); 135.1/135.3 (C-37); 130.1/131.1 (C-24);

130.1/129.3 (C-28); 123.9/123.7 (C-20); 16.7/116.7 (C-38); 98.7/98.0 (C-10); 96.3/97.8 (C-23).

#### Example 17: 33-p-Tolyloxythiocarbonyloxy-FK 506

[Formula I: R<sub>1</sub> = a group (c) wherein R<sub>6</sub> = OMe, R<sub>7</sub> = p-tolyloxythiocarbonyloxy; R<sub>2</sub> = OH, single bond in 23,24 position; R<sub>3</sub> = allyl; R<sub>4</sub> = OH, single bond in 10,11 position]

[Process variant e), acylation]

A solution of 2 g FK 506 in 70 ml of acetonitrile is successively reacted with 0.46 g 4-dimethylaminopyridine and 1.8 g p-tolyloxythiocarbonyl chloride and the mixture is stirred for 15 hours at room temperature. The reaction mixture is then diluted with acetic acid ethyl ester and successively washed with saturated aqueous sodium bicarbonate solution, 0.5 N hydrochloric acid and water, dried over sodium sulfate, filtered and concentrated under reduced pressure. The title compound is isolated from the residue as a light yellow foamy resin by column chromatography over silicagel (eluant: acetic acid ethyl ester / n-hexane 1:1):

<sup>1</sup>H-NMR: 7.22 and 7.01 (AABB-syst., ar-H); 5.35 (d, J=1 Hz, H-26); 5.18 (dxdxd, J=5 Hz, 9.5 Hz and 11 Hz, H-33); 3.475, 3.47, 3.41, 3.40, 3.355 and 3.32 (each s, -OCH<sub>3</sub>); 2.38 (s, ar-CH<sub>3</sub>);

#### Example 18: 33-Aminomethylcarbonyloxy-Δ<sup>23</sup>-FK 506

[Formula I: R<sub>1</sub> = a group (c) wherein R<sub>6</sub> = OMe, R<sub>7</sub> = R<sub>8</sub>R<sub>9</sub>CHCOO-(R<sub>8</sub> = amino; R<sub>9</sub> = H); R<sub>2</sub> = absent, double bond in 23,24 position; R<sub>3</sub> = allyl; R<sub>4</sub> = OH, single bond in 10,11 position]

[Process variant e)]

2 g N-BOC-glycine, 1 g dicyclohexylcarbodiimide, 0.5 g Δ<sup>23</sup>-FK 506 (second compound of Example 17 in EP 184162) and 1 g 4-dimethylaminopyridine are successively taken up at room temperature in 70 ml of acetonitrile and the mixture is stirred for 20 minutes at room temperature. The reaction mixture is filtered, the filtrate diluted with acetic acid ethyl ester and successively washed with 1 N hydrochloric acid, aqueous

sodium bicarbonate solution and water, the organic phase is dried over sodium sulfate, filtered, concentrated, and the residue is taken up in 50 ml of acetonitrile.

In order to split off the protecting group 0.5 g p-toluenesulfonic acid monohydrate is added and the mixture heated to refluxing for 5 minutes, the solution is cooled off, diluted with acetic acid ethyl ester, washed to neutrality with water, the organic phase is dried over sodium sulfate and concentrated. From the residue the **title compound** is obtained as a colourless foamy resin after column chromatography over silicagel (eluant: acetic acid ethyl ester / methanol 20:3):  
<sup>1</sup>H-NMR: about 6:5 mixture of conformers:  
 6.81 (dxd, J=5 Hz and 15 Hz) resp. 6.76 (dxd, J=75 Hz and 15 Hz) H-24; 6.18 (dxd, J=1 Hz and 15 Hz) resp. 6.29 (dxd, J=1 Hz and 15 Hz) H-23; 4.77 (m, H-33);

#### Example 19: 24-tert-Butyldimethylsilyloxy-FR 520-33-[(tert-butyldimethylsilyloxy)-(S)-lactate]

[Formula I: R<sub>1</sub> = a group (c) wherein R<sub>6</sub> = OMe, R<sub>7</sub> = R<sub>8</sub>R<sub>9</sub>CHCOO- (R<sub>8</sub> = OtBDMS, R<sub>9</sub> = Me, S-configuration); R<sub>2</sub> = OtBDMS, single bond in 23,24 position; R<sub>3</sub> = Et; R<sub>4</sub> = OH, single bond in 10,11 position]

[Process variant e)]

To a solution of 450 mg 24-tert-butyldimethylsilyloxy-FR 520 and 120 mg tert-butyldimethylsilyloxy-(S)-lactic acid in 10 ml of dichloromethane are added at room temperature 120 mg N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride and 23 mg dimethylaminopyridine. After 60 hours the reaction mixture is diluted with acetic acid ethyl ester, washed successively with 0.5 N hydrochloric acid and then water, dried over sodium sulfate, filtered, and the solvent is evaporated under reduced pressure. The residue is chromatographed over silicagel (eluant: n-hexane / acetic acid ethyl ester 2:1). The **title compound** is obtained as a colourless foam:

<sup>1</sup>H-NMR: 1.41 (d, J=7 Hz); 4.34 [q, J=7 Hz, -COCH(CH<sub>3</sub>)OSi]; 4.75 (m, H-33).

#### Example 20: FK 506-33-glycolate

[Formula I: R<sub>1</sub> = a group (c) wherein R<sub>6</sub> = OMe, R<sub>7</sub> = R<sub>8</sub>R<sub>9</sub>CHCOO- (R<sub>8</sub> = OH, R<sub>9</sub> = H); R<sub>2</sub> = OH, single bond in 23,24 position; R<sub>3</sub> = allyl; R<sub>4</sub> = OH, single bond in 10,11 position]

[Process variant e)]

To a solution of 300 mg tert-butyldimethylsilyloxymethylcarboxylic acid in 5 ml of dichloromethane are added under stirring at 0° 0.67 ml of oxalyl chloride and one drop of dimethylformamide. The mixture is brought to room temperature and is stirred for 1 hour. The reaction mixture is concentrated under reduced pressure. The residue is taken up in 5 ml of dichloromethane and this solution is added dropwise at 0° to a solution of 600 mg FK 506, 0.28 ml triethylamine and a catalytic quantity of 4-dimethylaminopyridine. After 18 hours stirring at 0° the solution is diluted with acetic acid ethyl ester, successively washed with 0.1 N hydrochloric acid and water, the organic phase is dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue is taken up in 20 ml of acetonitrile, reacted with 0.5 ml of 40 % wt. aqueous hydrofluoric acid and stirred for 20 minutes at room temperature. The mixture is diluted with acetic acid ethyl ester, washed with saturated aqueous sodium hydrogen carbonate solution, dried over sodium sulfate, filtered and concentrated under reduced pressure. The **title compound** is obtained as a colourless foamy resin from the residue by chromatography over silicagel (eluant: n-hexane / acetic acid ethyl ester):

<sup>1</sup>H-NMR: 4.13 (s, -COCH<sub>2</sub>OH); 4.41 (d, br, J=13 Hz, H-6e); 4.60 (d, br, J=4 Hz, H-2); 4.78 (m, H-33); 5.16 + 5.30 (H-26).

The following compounds of formula I are obtained in analogous manner in accordance with process variant e):

Analogous				Physicochemical							
Example No.	to Ex. No.	R <sub>1</sub>	R <sub>5</sub>	R <sub>6</sub>	R <sub>7</sub>	R <sub>2</sub>	Position 23,24	R <sub>3</sub>	R <sub>4</sub>	Position 10,11	characterization data
21	17 to 20	(c)	na	OMe	BOC-NHCH <sub>2</sub> COO-	OtBDS	sb	allyl	OH	sb	cfr; NMR*
22	17 to 20	(c)	na	OMe	tBDS-OCCH <sub>2</sub> COO-	OtBDS	sb	allyl	OH	sb	cfr; NMR*
23	17 to 20	(c)	na	OMe	BOC-NHCH <sub>2</sub> COO-	OtBDS	sb	Et	OH	sb	cfr
24	17 to 20	(c)	na	OMe	tBDS-OCCH <sub>2</sub> COO-	OtBDS	sb	Et	OH	sb	cfr; NMR*
25a	17 to 20	(c)	na	OMe	BOC-NHCH <sub>2</sub> COO-	OH	sb	allyl	OH	sb	cfr; NMR*
25b	17 to 20	(c)	na	OMe	OH	BOC-NHCH <sub>2</sub> COO-	sb	allyl	OH	sb	cfr; NMR*
25c	17 to 20	(c)	na	OMe	BOC-NHCH <sub>2</sub> COO-	BOC-NHCH <sub>2</sub> COO-	sb	allyl	OH	sb	cfr; NMR*
25d	17,19,20	(c)	na	OMe	BOC-NHCH <sub>2</sub> COO-	absent	db	allyl	OH	sb	cfr; NMR*
26a	17 to 20	(c)	na	OMe	BOC-NHCH <sub>2</sub> COO-	OH	sb	Et	OH	sb	cfr;
26b	17 to 20	(c)	na	OMe	OH	BOC-NHCH <sub>2</sub> COO-	sb	Et	OH	sb	cfr
26c	17 to 20	(c)	na	OMe	BOC-NHCH <sub>2</sub> COO-	BOC-NHCH <sub>2</sub> COO-	sb	Et	OH	sb	cfr
26d	17 to 20	(c)	na	OMe	BOC-NHCH <sub>2</sub> COO-	absent	db	Et	OH	sb	cfr
27a	17 to 20	(c)	na	OMe	NH <sub>2</sub> COCOO-	OH	sb	allyl	OH	sb	cfr
27b	17 to 20	(c)	na	OMe	NH <sub>2</sub> COCOO-	NH <sub>2</sub> COCOO-	sb	allyl	OH	sb	cfr; NMR*
27c	17 to 20	(c)	na	OMe	NH <sub>2</sub> COCOO-	absent	db	allyl	OH	sb	cfr
28a	17 to 20	(c)	na	OMe	NH <sub>2</sub> COCOO-	OH	sb	Et	OH	sb	cfr; NMR*
28b	17 to 20	(c)	na	OMe	NH <sub>2</sub> COCOO-	NH <sub>2</sub> COCOO-	sb	Et	OH	sb	cfr
28c	17 to 20	(c)	na	OMe	NH <sub>2</sub> COCOO-	absent	db	Et	OH	sb	cfr
29	17 to 20	(c)	na	OMe	NH <sub>2</sub> COCOO-	OtBDS	sb	Et	OH	sb	cfr; NMR*
30	17 to 20	(c)	na	OMe	NH <sub>2</sub> COCOO-	OtBDS	sb	allyl	OH	sb	cfr
31	17 to 20	(c)	na	OMe	p-tolylxy-thiocarbonyloxy	OH	sb	Et	OH	sb	cfr; NMR*
32	17 to 20	(c)	na	OMe	HOCH <sub>2</sub> COO-	OH	sb	Et	OH	sb	cfr; NMR*

Example No.	Analogous to Ex. No.	R <sub>1</sub>	R <sub>5</sub>	R <sub>6</sub>	R <sub>7</sub>	R <sub>2</sub>	Position 23,24	R <sub>3</sub>	R <sub>4</sub>	Position 10,11	Physicochemical characterization data
33	17 to 20	(c)	na	OMe	tEDMS-OCH(CH <sub>3</sub> )COO- (S)	OH	sb	Et	OH	sb	cfr
34	17 to 20	(c)	na	OMe	iBuoyloxy	OtEDMS	sb	Et	OH	sb	cfr; NMR*
35	17 to 20	(c)	na	OMe	iBuoyloxy	OtEDMS	sb	allyl	OH	sb	cfr
36	17 to 20	(c)	na	OMe	iBuoyloxy	OH	sb	allyl	OH	sb	cfr
37	17 to 20	(c)	na	OMe	iBuoyloxy	OH	sb	Et	OH	sb	cfr; NMR*
38	17,18,20	(c)	na	OMe	tEDMS-OCH(CH <sub>3</sub> )COO- (S)	OtEDMS	sb	Et	OH	sb	cfr; NMR*
* <sup>1</sup> H-NMR:	Example 21:	mixture of conformers:									
<sup>1</sup> H-NMR:	Example 22:	4.85 (m, H-33); 3.93 (s, O=C-CH <sub>2</sub> -N-); 3.22 (m, H-32);									
<sup>1</sup> H-NMR:	Example 24:	4.25 (s, -COCH <sub>2</sub> OSi); 4.76 (m, H-33);									
<sup>1</sup> H-NMR:	Example 25a:	4.26 (s, -COCH <sub>2</sub> OSi);									
<sup>1</sup> H-NMR:	Example 25b:	5.7 (m, H-37); 4.75 (dx dx d, J=5 Hz, 9 Hz and 10 Hz, H-33); 3.93 (m, N-CH <sub>2</sub> -); 1.46 (s, tBu);									
<sup>1</sup> H-NMR:	Example 25c:	mixture of conformers:									
<sup>1</sup> H-NMR:	Example 27b:	5.69 (m, H-37); 4.52 (H-2); 4.44 (H-6 eq.); 3.87 (m, -N-CH <sub>2</sub> -C=O); 1.46 (s, N-BOC);									
<sup>1</sup> H-NMR:	Example 28a:	5.7 (m, H-37); 4.76 (dx dx d, J=5 Hz, 8 Hz and 10 Hz, H-33); 3.93/3.87 (m/m, -N-CH <sub>2</sub> ); 1.46 (s, tBu);									
<sup>1</sup> H-NMR:	Example 29:	about 2:1 mixture of conformers:									
<sup>1</sup> H-NMR:	Example 29a:	7.15-7.0 and 6.1-6.2 (b, each 2H, O=C-NH <sub>2</sub> ); 5.28 and 5.42 (q/q, J=5 and 5 Hz, H-24); 4.84 (m, H-33);									
<sup>1</sup> H-NMR:	Example 29b:	mixture of conformers:									
<sup>1</sup> H-NMR:	Example 29c:	7.01 and 5.98 (-CONH <sub>2</sub> ); 5.35 (d, J=1 Hz, H-26); 4.85 (m, H-33); 4.61 (db, J=3 Hz, H-2);									
<sup>1</sup> H-NMR:	Example 29d:	4.44 (db, J=13 Hz, H-6 eq.);									
<sup>1</sup> H-NMR:	Example 29e:	mixture of conformers:									
<sup>1</sup> H-NMR:	Example 29f:	7.03 and 6.12 (CONH <sub>2</sub> ); 4.85 (m, H-33); 4.45 (H-2); 4.42 (H-6 eq.); 0.88 (s, tBu); 0.04 (s, Si-CH <sub>3</sub> );									

- <sup>1</sup>H-NMR: Example 31: mixture of conformers:  
7.22/7.02 (AA'/BB'-syst., ar-H); 5.35 (d, J=1 Hz, H-26); 5.18 (m, H-33); 4.63 (db, J=4 Hz, H-2); 4.45 (db, J=13 Hz, H-6 eq.); 2.37 (s, ar-CH<sub>3</sub>);
- <sup>1</sup>H-NMR: Example 32: 4.13 (s, -COCH<sub>2</sub>OH); 4.40 (d, br, J=13 Hz, H-6e); 4.58 (d, br, J=4 Hz, H-2); 4.78 (m, H-33); 5.17 + 5.30 (H-26);
- <sup>1</sup>H-NMR: Example 34: 4.69 (m, H-33); 2.55 [septet, J=7 Hz, O=C-CH(CH<sub>3</sub>)<sub>2</sub>]; 1.17 [d, J=7 Hz, C(CH<sub>3</sub>)<sub>2</sub>];
- <sup>1</sup>H-NMR: Example 37: 4.68 (dxdxd, J=5 Hz, 10 Hz and 11 Hz, H-33); 2.55 [septet, J=7 Hz, O=C-CH(CH<sub>3</sub>)<sub>2</sub>]; 1.17 [d, J=7 Hz, C(CH<sub>3</sub>)<sub>2</sub>];
- Minor component: 1.23 (d, J=7 Hz; 4.30 [dq, J<sub>1</sub>=5 Hz, J<sub>2</sub>=7 Hz, -COCH(CH<sub>3</sub>)OH]; 4.78 (ddd, J<sub>1</sub>=5 Hz, J<sub>2</sub>=9 Hz, J<sub>3</sub>=11 Hz, H-33); 5.20 (H-26);
- <sup>1</sup>H-NMR: Example 38: see Example 19.

**Example 39: 24-tert-Butyldimethylsilyloxy-33-aminoxyloxy-FK 506**

5

[Formula I:  $R_1 = a$  group (c) wherein  $R_6 = OMe$ ,  $R_7 = \text{aminoxyloxy}$ ;  $R_2 = OtBDMS$ , single bond in 23,24 position;  $R_3 = \text{allyl}$ ;  $R_4 = OH$ , single bond in 10,11 position]

10

[Process variant f), treatment with oxalyl chloride and ammonia]

A solution of 24,33-bis-(tert-butyldimethylsilyloxy)-FK 506 in 70 ml of acetonitrile is reacted at 0° to 5° with 1 ml of oxalyl chloride and stirred at 0 to 5° for 40 minutes. The reaction mixture is stirred with a mixture of acetic acid ethyl ester and saturated aqueous ammonia solution, any precipitate formed is sucked off, the phases are separated, the organic phase is washed successively with 1 N hydrochloric acid and then water, dried over sodium sulfate, filtered and concentrated under reduced pressure. From the residue the **title compound** is obtained as a colourless foamy resin following column chromatography over silicagel (eluant: n-hexane / acetic acid ethyl ester 1:1):

15 <sup>1</sup>H-NMR: about 2:1 mixture of conformers:

7.04 and 6.17 (b, each 1 H,  $H_2NC=O$ ); 4.86 (m, H-33).

25

30

35

40



The following compounds of formula I are obtained in analogous manner in accordance with process variant f):											
Example No.	Analogous to Ex. No.	R <sub>1</sub>	R <sub>5</sub>	R <sub>6</sub>	R <sub>7</sub>	R <sub>2</sub>	Position 23,24	R <sub>3</sub>	R <sub>4</sub>	Position 10,11	Physicochemical characterization data
40	39 <sup>1)</sup>	(c)	na	OMe	NH <sub>2</sub> COCOO-	NH <sub>2</sub> COCOO-	sb	allyl	OH	sb	cfr; NMR*
41	39,49	(c)	na	OMe	NH <sub>2</sub> COCOO-	OTBDMS	sb	Et	OH	sb	cfr; NMR*
42	39,40	(c)	na	OMe	NH <sub>2</sub> COCOO-	NH <sub>2</sub> COCOO-	sb	Et	OH	sb	cfr

<sup>1)</sup> Stirring is effected for 1 hour at room temperature; column chromatography is effected using an eluant gradient of 3:1 to 1:3; <sup>1</sup>H-NMR: Example 40: see Example 27b; Example 41: see Example 29.

<sup>1)</sup> Stirring is effected for 1 hour at room temperature; column chromatography is effected using an eluant gradient of 3:1 to 1:3; <sup>1</sup>H-NMR: Example 40: see Example 27b; Example 41: see Example 29.

**Example 43: 24-Methoxy-33-tert-butyldimethylsilyloxy-FK 506**

[Formula I:  $R_1$  = a group (c) wherein  $R_6$  = OMe,  $R_7$  = OtBDMS;  $R_2$  = OMe, single bond in 23,24 position;  $R_3$  = allyl;  $R_4$  = OH, single bond in 10,11 position]

[Process variant g), methylation]

1 g 33-tert-butyldimethylsilyloxy-FK 506 is dissolved into a mixture of 50 ml of dichloromethane and 0.04 ml of borontrifluoride etherate previously cooled to 0° to 5°. A solution of 20 ml of an approximately 1 N solution of diazomethane in methylene chloride is then added dropwise in such a manner that the yellow coloration of the solution which initially forms persists for as shortly as possible. The reaction mixture is diluted with acetic acid ethyl ester, successively washed with saturated aqueous sodium hydrogen carbonate solution and water, dried over sodium sulfate, filtered and the solvent is removed under reduced pressure. The title compound is obtained as a colourless foamy resin from the residue following column chromatographic purification over silicagel (eluant: acetic acid ethyl ester / n-hexane):

<sup>1</sup>H-NMR: about 3:1 mixture of conformers:

main conformer: 5.25 (d, J=8 Hz, H-29); 5.17 (d, J=7 Hz, H-26); 4.79 (d, J=10 Hz, H-20); 3.82 (dxd, J=9 Hz and 1.5 Hz, H-14); 3.42, 3.40, 3.33 and 3.24(4xs, OCH<sub>3</sub>); 2.68 (dxd, J=13 Hz and 8 Hz, H-23);

minor conformer: 3.90 (dxd, J=9/2,5 Hz, H-14);

The following compounds of formula I are obtained in analogous manner in accordance with process variant g):

Example No.	Analogous to Ex. No.	$R_1$	$R_5$	$R_6$	$R_7$	$R_2$	Position 23,24	$R_3$	$R_4$	Position 10,11	Physicochemical characterization data
44	43 <sup>1)</sup>	(c)	na	OMe	OMe	OtBDMS	sb	allyl	OH	sb	cfr; NMR*

\*<sup>1</sup>H-NMR: about 2:1 mixture of conformers: main conformer: 5.22 (d, J=7Hz, H-26); 4.84 (d, J=10Hz, H-20); 4.07 (m, H-24); 3.80 (dxd, J=9Hz and 1.5Hz, H-14); 3.45, 3.44, 3.40 and 3.32 (4xs, OCH<sub>3</sub>); 2.78 (dxd, J=15Hz and .5Hz, H-23); 0.87 (tBu); minor conformer: 4.26 (m, H-24); 3.94 (dxd, J=9Hz and 2.5Hz, H-14); 0.86 (tBu);

<sup>1)</sup> Starting from 24-tert-butyldimethylsilyloxy-FK 506.

**Example 45: 24-tert-Butyldimethylsilyloxy-33-oxo-FR 520**

[Formula I:  $R_1$  = a group (c) wherein  $R_6$  = OMe,  $R_7$  = oxo;  $R_2$  = OtBDMS, single bond in 23,24 position;  $R_3$  = Et;  $R_4$  = OH, single bond in 10,11 position]

[Process variant h), oxidation]

2 g 24-tert-butyldimethylsilyloxy-FR 520 and 1 g N-methylmorpholin-N-oxide are dissolved in 100 ml of methylene chloride, reacted with 5 g molecular sieve (Molsieb 4A) and the mixture is stirred for 15 minutes at room temperature. 0.15 g tetrapropylammonium perruthenate is added and stirring is continued for 3 more hours at room temperature. The mixture is concentrated, the residue is taken up in acetic acid ethyl ester and the solution successively washed with saturated aqueous sodium hydrogen sulfite solution, saturated aqueous sodium chloride and saturated aqueous copper sulfate solution, the organic phase is dried over sodium sulfate, filtered and concentrated under reduced pressure. The title compound is

obtained from the residue following column chromatography over silicagel (eluant: n-hexane / acetic acid ethyl ester).

5 The following compounds of formula I are obtained in analogous manner in accordance with process variant h):

Example No.	Analogous to Ex. No.	R <sub>1</sub>	R <sub>5</sub>	R <sub>6</sub>	R <sub>7</sub>	R <sub>2</sub>	Position 23,24	R <sub>3</sub>	R <sub>4</sub>	Position 10,11	Physicochemical characterization data
10 46	45 <sup>1)</sup>	(c)	na	OMe	OtBDMS	oxo	sb	Et	OH	sb	cfr
46a	45 <sup>2)</sup>	(c)	na	OMe	oxo	OtBDMS	sb	allyl	OH	sb	cfr; NMR*
46b	45	(c)	na	OMe	OtBDMS	oxo	sb	allyl	OH	sb	cfr; NMR*

\* Example 46a: <sup>13</sup>C-NMR: see Example 15a; Example 46b: <sup>1</sup>H-NMR and <sup>13</sup>C-NMR: see Example 16b;

15 <sup>1)</sup> Starting from 33-tert-butyldimethylsilyloxy-FR 520 (DOS 39 38 754);

<sup>2)</sup> Starting from 24-tert-butyldimethylsilyloxy-FK 506;

<sup>3)</sup> Starting from 33-tert-butyldimethylsilyloxy-FK 506;

The compounds of Examples 47 and 50 may be prepared in analogous manner according to process variant h).

#### 25 Example 47: 24-Oxo-FK 506

[Formula I: R<sub>1</sub> = a group (c) wherein R<sub>6</sub> = OMe, R<sub>7</sub> = OH; R<sub>2</sub> = oxo, single bond in 23,24 position; R<sub>3</sub> = allyl; R<sub>4</sub> = OH, single bond in 10,11 position]

[Process variant deprotection]

3.6 g 24-oxo-33-tert-butyldimethylsilyloxy-FK506 (compound of Example 16b) is dissolved at room temperature into a mixture of 110 ml of acetonitrile and 3 ml of 40 % wt. aqueous hydrofluoric acid and the mixture is stirred at room temperature for 45 minutes. The reaction mixture is diluted with acetic acid ethyl ester, washed successively with saturated aqueous sodium bicarbonate solution and then water, dried over sodium sulfate, filtered and concentrated under reduced pressure. The title compound is obtained as a colourless foamy resin following chromatographic purification of the residue over silicagel (eluant: acetic acid ethyl ester / n-hexane 3:2):

<sup>1</sup>H-NMR: about 1:1 mixture of conformers:

5.80 and 5.60 (s, H-23); 3.44, 3.41, 3.39, 3.38 and 2x3.275 (OCH<sub>3</sub>).

The following compounds of formula I are obtained in analogous manner in accordance with process variant deprotection:

Example No.	Analogous to Ex. No.	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	Position 23,24	R <sub>5</sub>	R <sub>6</sub>	R <sub>7</sub>	R <sub>8</sub>	Position 10,11	Physicochemical characterization data
48	471)	(c) na	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	cfr; NMR*
49	472)	(c) na	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	cfr; NMR*
50	473)	(c) na	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	cfr; NMR*
51	474)	(c) na	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	cfr; NMR*
52	475)	(c) na	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	cfr
53	476)	(c) na	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	cfr; NMR*
54	477)	(c) na	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	cfr; NMR*
55	478)	(c) na	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	cfr; NMR*
56	479)	(c) na	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	cfr
57	4710)	(c) na	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	cfr
58	4711)	(c) na	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	cfr
59	4712)	(c) na	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	cfr
60	4713)	(c) na	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	cfr; NMR*
61	4714)	(c) na	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	cfr; NMR*
62	4715)	(c) na	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	cfr; NMR*
63	4716)	(c) na	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	cfr

Example No.	Analogous				R <sub>7</sub>	R <sub>2</sub>	Position 23,24	R <sub>3</sub>	R <sub>4</sub>	Physicochemical characterization data	
	Ex. No.	R <sub>1</sub>	R <sub>5</sub>	R <sub>6</sub>						10,11	
64	4717)	(a)	I	OMe	na	OH	sb	Et	OH	sb	..
65a	4718)	(a)	Cl	OMe	na	OH	sb	allyl	OH	sb	cfr; NMR*
65b	4718)	(a)	Cl	OMe	na	OH	sb	allyl	absent	db	cfr
66a	4719)	(a)	Cl	OMe	na	OH	sb	Et	OH	sb	cfr; NMR*
66b	4719)	(a)	Cl	OMe	na	OH	sb	Et	absent	db	cfr; NMR*
67a	4720)	(a)	Br	OMe	na	OH	sb	Et	OH	sb	cfr; NMR*
67b	4720)	(a)	Br	OMe	na	OH	sb	Et	absent	db	cfr
68a	4721)	(a)	N <sub>3</sub>	OMe	na	OH	sb	allyl	OH	sb	cfr; NMR*
68b	4721)	(a)	N <sub>3</sub>	OMe	na	OH	sb	allyl	absent	db	cfr; NMR*
69a	4722)	(a)	Br	OMe	na	OH	sb	allyl	OH	sb	cfr
69b	4722)	(a)	Br	OMe	na	OH	sb	allyl	absent	db	cfr
70a	4723)	(a)	N <sub>3</sub>	OMe	na	OH	sb	Et	OH	sb	cfr; NMR*
70b	4723)	(a)	N <sub>3</sub>	OMe	na	OH	sb	Et	absent	db	cfr

- 1) Starting from the compound of Example 25a;
- 2) Starting from the compound of Example 25d;
- 3) Starting from the compound of Example 46 (-16d);
- 4) Starting from the compound of Example 23;
- 5) Starting from the compound of Example 26d;
- 6) Starting from the compound of Example 22;
- 7) Starting from the compound of Example 24;
- 8) Starting from the compound of Example 19 or of Example 33;
- 9) Starting from the compound of Example 26b;
- 10) Starting from the compound of Example 26c;

- 11) Starting from the compound of Example 25c;
- 12) Starting from the compound of Example 25b;
- 13) Starting from the compound of Example 25a;
- 14) Starting from the compound of Example 26a;
- 15) Starting from the compound of Example 34;
- 16) Starting from the compound of Example 35;
- 17) Starting from the compound of Example 6a;
- 18) Starting from the compound of Example 1;
- 19) Starting from the compound of Example 3;
- 20) Starting from the compound of Example 4;
- 21) Starting from the compound of Example 2;
- 22) Starting from the compound of Example 5;
- 23) Starting from the compound of Example 6;

- \*NMR: Example 48: <sup>1</sup>H-NMR: about 2:1 mixture of conformers:  
 5.33 and 5.20 (d/d, J=1 Hz and 1 Hz, H-26); 4.84 (dxdxd, J=5 Hz, 9.5 Hz and 11 Hz, H-33); 3.44 (s, 2H, O=C-CH<sub>2</sub>-N-); 3.22 (dxdxd, J=5 Hz, 9.5 Hz and 11 Hz, H-32);
- Example 49: <sup>1</sup>H-NMR: see Example 18;
- Example 50: <sup>1</sup>H-NMR: mixture of conformers:  
 5.8 and 5.6 (s/s, H-23); 5.69 (H-26); 4.38 (d, J=13 Hz, H-6e); 4.19 (t, H-2); 3.80 (dxd, J=9 Hz and 2 Hz, H-14);
- Example 51: <sup>1</sup>H-NMR: about 2:1 mixture of conformers:  
 5.34 (d, J=2 Hz, H-26); 4.75 (dxdxd, J=5 Hz, 9 Hz and 10 Hz, H-33); 4.61 (db, J=4 Hz, H-2); 4.44 (db, J=13 Hz, H-6e); 3.45 (s, -CH<sub>2</sub>-N);

- Example 53: <sup>1</sup>H-NMR: see Example 20;  
 Example 54: <sup>1</sup>H-NMR: see Example 32;  
 Example 55: <sup>1</sup>H-NMR: mixture of conformers: main conformer: 1.23 (d, J=7 Hz); 4.30 [dq, J<sub>1</sub>=5 Hz, J<sub>2</sub>=7 Hz, -COCH(CH<sub>3</sub>)OH]; 4.44 (d, br, J=13 Hz, H-6e); 4.61 (d, br, J=4 Hz); 4.78 (ddd, J<sub>1</sub>=5 Hz, J<sub>2</sub>=5 Hz, J<sub>3</sub>=11 Hz, H-33); 5.34 (H-26);  
 Example 60: <sup>1</sup>H-NMR: see Example 48;  
 Example 61: <sup>1</sup>H-NMR: see Example 51;  
 Example 62: <sup>1</sup>H-NMR: see Example 37;  
 Example 65a: <sup>1</sup>H-NMR: 4.59 (m, H-33);  
<sup>13</sup>C-NMR: about 2:3 mixture of conformers:  
 main conformer: 59.1 (C-33); 79.2 (C-32); 97.5 (C-10); 116.4 (C-38); 123.0 (C-20); 135.6 (C-37); 138.4 (C-19); 164.6 (C-8); 168.9 (C-1); 196.4 (C-9); 209.4 (C-22);  
 4.56 (m, H-33);  
 2.09 (s, 11-CH<sub>3</sub>); 4.5 (bm, H-33);  
 about 2:1 mixture of conformers:  
 main conformer: 56.2 (C-33); 80.6 (C-32); 116.4 (C-38); 122.9 (C-20); 124.8 (C-11); 129.5 (C-29); 131.9 (C-28); 135.8 (C-37); 140.0 (C-19); 142.9 (C-10); 166.7 (C-8); 168.7 (C-1); 188.0 (C-9); 212.4 (C-22);  
 minor conformer: 56.1 (C-33); 80.6 (C-32); 116.5 (C-38); 123.6 (C-20); 126.4 (C-11); 128.5 (C-29); 131.8 (C-28); 135.6 (C-37); 137.4 (C-19); 144.1 (C-10); 166.5 (C-8); 169.5 (C-1); 184.8 (C-9); 213.3 (C-22);  
 4.44 (d, J=13 Hz, H-6 eq.); 4.60 (d, J=4 Hz, H-2); 4.70 (sb, H-33);  
 4.07 (m, v<sub>1/2</sub> = 8 Hz, H-33);  
 about 2:1 mixture of conformers: 4.06 (m, H-33); 2.09 and 1.94 (2s, 11-CH<sub>3</sub>);  
 about 5:4 mixture of conformers: 5.60 resp. 5.79 (s resp. s, H-23); 5.70 resp. 5.66 (d, J=3 Hz resp. d, J=3 Hz, H-26); 4.38 (d, J=13 Hz, H-6e); 4.15 (t, H-2);  
 3.80 (dxd, J=9 Hz and 2 Hz, H-14).  
 \*\* Iodine analysis: theor.: 14.06 %; found: 13.57 %.
- Example 67a: <sup>1</sup>H-NMR:  
 Example 68a: <sup>1</sup>H-NMR:  
 Example 68b: <sup>1</sup>H-NMR:  
 Example 70a: <sup>1</sup>H-NMR:

The compounds of Examples 10a, 11a, 12, 13, 27a and 28a may be prepared in analogous manner according to process variant deprotection.

Example 71: 24-tert-Butyldimethylsilyloxy-29-des-(4-hydroxy-3-methylcyclohexyl)-29-(3-formylcyclopentyl)-FR 520

[Formula I:  $R_1$  = a group (d);  $R_2$  = OtBDMS, single bond in 23,24 position;  $R_3$  = Et;  $R_4$  = OH, single bond in 10,11 position]

[Process variant protection]

A solution of 1.2 g 29-des-(4-hydroxy-3-methylcyclohexyl)-29-(3-formylcyclopentyl)-FR 520 - (compound of Example 12), 1.5 g tert-butyldimethylsilyl chloride and 0.8 g imidazole in 20 ml of dry dimethylformamide is stirred for 15 hours at room temperature and thereafter partitioned between 1 N hydrochloric acid solution and acetic acid ethyl ester. The organic phase is separated, washed with saturated aqueous sodium chloride solution, dried over sodium sulfate, filtered and concentrated under reduced pressure. The title compound is obtained from the residue as a colourless foamy resin following column chromatography over silicagel (eluant: n-hexane / acetic acid ethyl ester):  
<sup>1</sup>H-NMR: mixture of rotamers: 9.65 (d, J = 2 Hz, CHO); 5.39 (d, J = 9 Hz, H-29); 5.01 (d, J = 7.5 Hz, H-26); 4.81 (d, J = 10 Hz, H-20); 3.82 (dxd, J = 9/2 Hz, H-24).

The compounds of Examples 1 to 9, 16a to 16d, 19, 21 to 26d, 29, 30, 34, 35, 38, 39, 41 and 43 to 46b may be prepared in analogous manner according to process variant protection.

The compounds of the invention possess pharmacological activity. They are indicated for use as pharmaceuticals.

In particular they possess antiinflammatory, and immunosuppressant and antiproliferative activity.

Antiinflammatory activity may e.g. be determined in the following test methods:

1. Oxazolone allergic contact dermatitis in the mouse in vivo upon topical application: the test method is as described in F.M. Dietrich and R. Hess, *Int. Arch. Allergy* 38 (1970) 246-259.

The compounds elicit in this test an activity between about 15 % and about 68 % upon topical administration at a concentration of about 0.01 %.

2. DNFB allergy (swine): the test method is as described in e.g. EP 315978.

Topical application of a 1.2 % formulation of the compounds repeated twice results in from about 36 % to about 40 % inhibition of the inflammatory reaction.

Immunosuppressant and antiproliferative activity may e.g. be determined in the following test methods:

1. Proliferative response of lymphocytes to allogeneic stimulation in the mixed lymphocyte reaction (MLR) in vitro: T. Meo, "The MLR in the Mouse", *Immunological Methods*, L. Lefkovits and B. Pernis, Eds., Academic Press, N.Y. (1979), 227-239.

The compounds elicit in this test ( $IC_{50}$ ) suppression of mixed lymphocytes at a dosage of from about < 0.0008 µg/ml to about 0.09 µg/ml.

2. Inhibition of the primary humoral immune response to sheep erythrocytes in vitro: the test method is as described in R.I. Mishell and R.W. Dutton, *Science* 153 (1966) 1004-1006; R.I. Mishell and R.W. Dutton, *J. Exp. Med.* 126 (1967) 423-442.

The compounds are active in this test with an  $IC_{50}$  of from about 0.0024 µg/ml to about 0.32 µg/ml.

3. Inhibition of proliferation of human keratinocytes: the test method is as described in e.g. EP 315978.

The compounds are active in this test at concentrations of from about 1 µg/ml to about 10 µg/ml resulting in a inhibition of from about 30 % to about 90 %.

The compounds of the invention in free form and where such forms exist in pharmaceutically acceptable salt form are therefore indicated as antiinflammatory and as immunosuppressant and antiproliferative agents for use in the prevention and treatment of inflammatory conditions and of conditions requiring immunosuppression, such as

a) the prevention and treatment of

- resistance in situations of organ or tissue transplantation, e.g. of heart, kidney, liver, bone marrow and skin,

- graft-versus-host disease, such as following bone marrow grafts,

- autoimmune diseases such as rheumatoid arthritis, systemic Lupus erythematosus, Hashimoto's thyroiditis, multiple sclerosis, Myasthenia gravis, diabetes type I and uveitis,



- cutaneous manifestations of immunologically-mediated illnesses;
- b) the treatment of inflammatory and hyperproliferative skin diseases, such as psoriasis, atopic dermatitis, contact dermatitis and further eczematous dermatitises, seborrhoeic dermatitis, Lichen planus, Pemphigus, bullous Pemphigoid, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Lupus erythematosus and acne; and
- c) Alopecia areata.

The compounds may be administered systemically or topically.

For these indications the appropriate dosage will, of course, vary depending upon, for example, the host, the mode of administration and the nature and severity of the condition being treated. However, in general, satisfactory results are indicated to be obtained systemically at daily dosages of from about 0.15 mg/kg to about 1.5 mg/kg animal body weight. An indicated daily dosage in the larger mammal is in the range from about 0.01 mg to about 100 mg, conveniently administered, for example, in divided doses up to four times a day or in retard form.

For topical use satisfactory results are obtained with local administration of a 1-3 % concentration of active substance several times daily, e.g. 2 to 5 times daily. Examples of indicated galenical forms are lotions, gels and creams.

The compounds of the invention may be administered by any conventional route, in particular enterally, e.g. orally, e.g. in the form of tablets or capsules, or topically, e.g. in the form of lotions, gels or creams.

Pharmaceutical compositions comprising a compound of the invention in association with at least one pharmaceutical acceptable carrier or diluent may be manufactured in conventional manner by mixing with a pharmaceutically acceptable carrier or diluent. Unit dosage forms contain, for example, from about 0.0025 mg to about 50 mg of active substance.

Topical administration is e.g. to the skin. A further form of topical administration is to the eye, for the treatment of immune-mediated conditions of the eye, such as: auto-immune diseases, e.g. uveitis, keratoplasty and chronic keratitis; allergic conditions, e.g. vernal conjunctivitis; inflammatory conditions and corneal transplants, by the topical administration to the eye surface of a compound of the invention in a pharmaceutically acceptable ophthalmic vehicle.

The ophthalmic vehicle is such that the compound is maintained in contact with the ocular surface for a sufficient time period to allow the compound to penetrate the corneal and internal regions of the eye, e.g. the anterior chamber, posterior chamber, vitreous body, aqueous humor, vitreous humor, cornea, iris/ciliary, lens, choroid/retina and sclera.

The pharmaceutically acceptable ophthalmic vehicle may be e.g. an ointment, vegetable oil, or an encapsulating material.

Whilst the antiinflammatory and immunosuppressant and antiproliferative activity is the main activity of the compounds of the invention they also possesses some degree of activity in increasing sensitivity to, or in increasing the efficacy of, chemotherapeutic drug therapy.

This activity may e.g. be determined according to the test methods described in EP 360760.

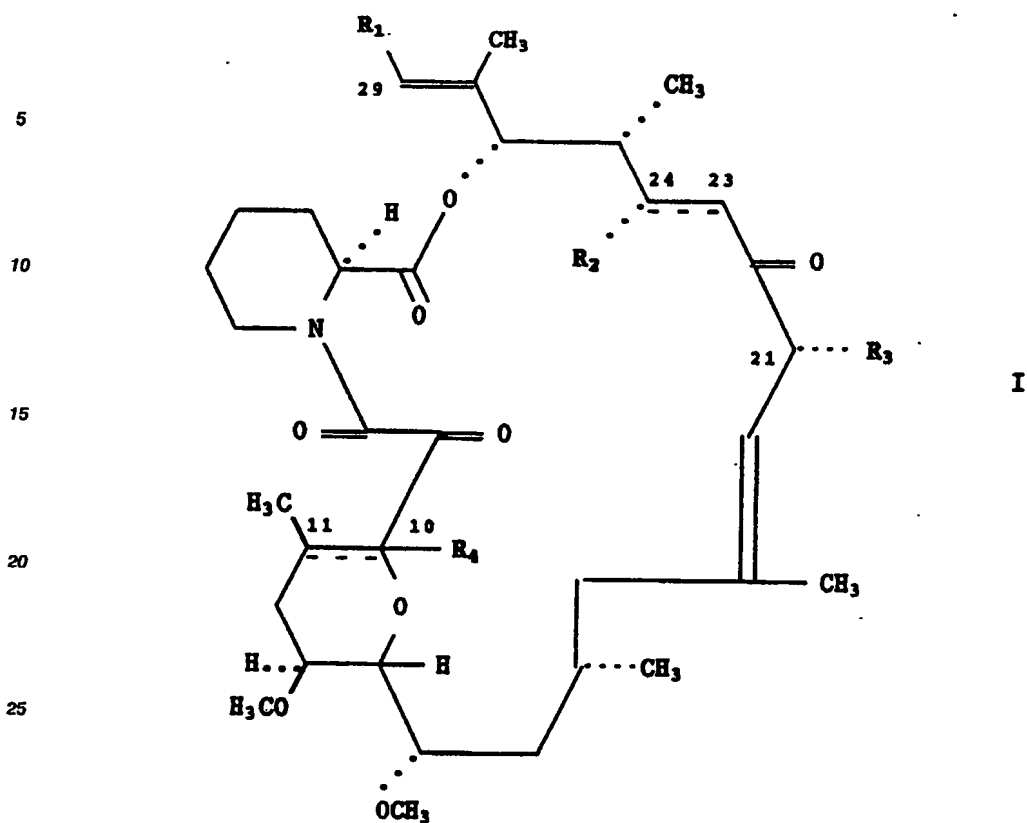
The compounds of the invention are therefore indicated for use in reversing chemotherapeutic drug resistance of varying types, e.g. acquired or innate, or in increasing sensitivity to administered drug therapy, e.g. as a means of reducing regular chemotherapeutic dosage levels, for example in the case of anti-neoplastic or cytostatic drug therapy, as a means of decreasing overall drug toxicity and, more especially, as a means of reversing or reducing resistance, including both inherent and acquired resistance, to chemotherapy.

Preferred in the above indications are the following compounds of the invention:

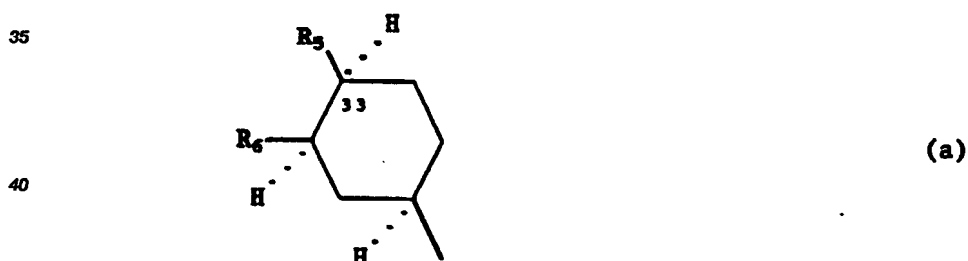
- 29-des-(4-hydroxy-3-methoxycyclohexyl)-29-(3-formylcyclopentyl)-FR 520 (compound of Example 12);
- 33-aminoxyloxy-FR 520 (compound of Example 28a);
- FR 520-33-glycolate (compound of Examples 32 and 54);
- 33-isobutanoyloxy-FR 520 (compound of Examples 37 and 62); and
- 33-epi-33-chloro-PR 520 (compound of Example 66a).

## Claims

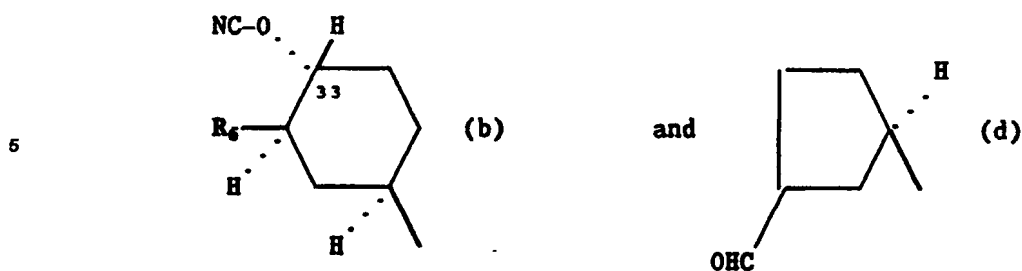
1. A compound of formula I



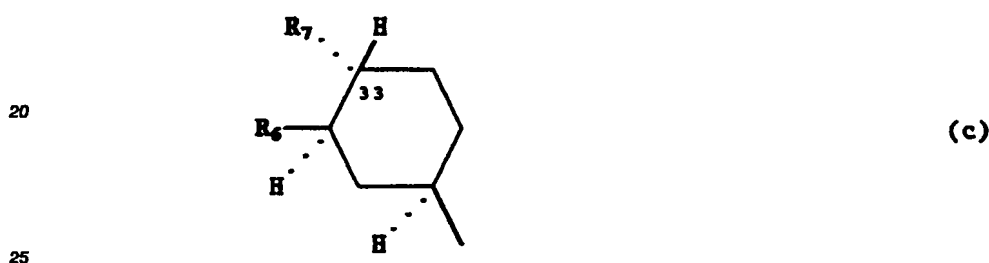
wherein  
either R<sub>1</sub> is a group (a) of formula



- 45 wherein R<sub>5</sub> is chloro, bromo, iodo or azido and R<sub>6</sub> is hydroxy or methoxy;  
R<sub>2</sub> is oxo and there is a single bond in 23,24 position; optionally protected hydroxy and there is a single or  
a double bond in 23,24 position; or absent and there is a double bond in 23,24 position; and  
R<sub>4</sub> is hydroxy and there is a single bond in 10,11 position; or absent and there is a double bond in 10,11  
position;  
50 or R<sub>1</sub> is a group (b) or (d) of formula



wherein  $R_6$  is as defined above;  
 $R_2$  is as defined above; and  
 $R_4$  is hydroxy and there is a single bond in 10,11 position;  
 15 or  $R_1$  is a group (c) of formula



wherein  $R_6$  is as defined above and  
 $R_7$  is oxo; optionally protected hydroxy; methoxy; methylthiomethoxy; isobutanoyloxy; aminooxalyloxy;  
 $R_8R_9CHCOO^-$  wherein  $R_8$  is optionally protected hydroxy or optionally protected amino and  $R_9$  is hydrogen  
 30 or methyl; or p-tolyloxythiocarbonyloxy;  
 $R_2$  is oxo and there is a single bond in 23,24 position; absent and there is a double bond in 23,24 position;  
 or is optionally protected hydroxy, methoxy, methylthiomethoxy, isobutanoyloxy, aminooxalyloxy or  $R_8R_9CH$   
 $COO^-$  wherein  $R_8$  and  $R_9$  are as defined above, and there is a single or a double bond in 23,24 position;  
 whereby for group (c)

- 35 1) when  $R_7$  is oxo, unprotected hydroxy or methoxy  
 then  $R_2$  is other than absent and other than unprotected hydroxy or methoxy, and  
 there is a single bond in 23,24 position;  
 2) when  $R_6$  is methoxy and  $R_7$  is methylthiomethoxy  
 then  $R_2$  is other than absent and other than unprotected hydroxy;  
 40 3) when  $R_6$  is methoxy and  $R_7$  is protected hydroxy  
 then  $R_2$  is other than optionally protected hydroxy; and  
 4) when  $R_6$  is hydroxy  
 then  $R_7$  is other than optionally protected hydroxy; and  
 $R_4$  is hydroxy and there is a single bond in 10,11 position; and  $R_3$  is methyl, ethyl, n-propyl or allyl;  
 45 in free form or, where such forms exist, in salt form.  
 2. A compound according to claim 1 which is a compound  $Ip_1$ ,  
 i.e. a compound of formula I wherein  
 $R_1$  is a group (a) wherein  $R_6$  is methoxy and  
 either  $R_5$  is chloro or bromo and  
 50  $R_4$  is hydroxy and there is a single bond in 10,11 position  
 or  $R_5$  is azido and  
 $R_4$  is hydroxy and there is a single bond in 10,11 position or absent and there is a double bond in 10,11  
 position;  
 $R_2$  is optionally protected hydroxy and there is a single or a double bond in 23,24 position; and  
 55  $R_3$  is as defined in claim 1;  
 in free form or, where such forms exist, in salt form.  
 3. A compound according to claim 1 which is a compound  $Ip_2$ ,  
 i.e. a compound of formula I wherein

R<sub>1</sub> is a group (c) wherein R<sub>6</sub> is methoxy and R<sub>7</sub> is oxo; optionally protected hydroxy; methoxy; methylthiomethoxy; aminooxalyloxy; R<sub>8</sub>CH<sub>2</sub>COO<sup>-</sup> wherein R<sub>8</sub> is optionally protected amino; or p-tolylox-ythiocarbonyloxy;

5 R<sub>2</sub> is absent and there is a double bond in 23,24 position; or optionally protected hydroxy, methoxy, methylthiomethoxy or aminooxalyloxy and there is a single or double bond in 23,24 position; whereby

1) when R<sub>7</sub> is oxo, unprotected hydroxy or methoxy

then R<sub>2</sub> is other than absent and other than unprotected hydroxy or methoxy, and there is a single bond in 23,24 position;

10 2) when R<sub>7</sub> is methylthiomethoxy

then R<sub>2</sub> is other than absent and other than unprotected hydroxy; and

3) when R<sub>7</sub> is protected hydroxy

then R<sub>2</sub> is other than optionally protected hydroxy; and

R<sub>4</sub> is hydroxy and there is a single bond in 10,11 position; and

15 R<sub>3</sub> is as defined in claim 1;

in free form or, where such forms exist, in salt form.

4. A compound according to claim 1 which is a **compound Ip<sub>3</sub>**,

i.e. a compound of formula I wherein

R<sub>1</sub> is a group (b) wherein R<sub>6</sub> is methoxy,

20 R<sub>2</sub> is optionally protected hydroxy and there is a single bond in 23,24 position; or absent and there is a double bond in 23,24 position;

R<sub>4</sub> is hydroxy and there is a single bond in 10,11 position; and

R<sub>3</sub> is as defined in claim 1;

in free form or, where such forms exist, in salt form.

25 5. A compound according to claim 1 which is a **compound Ip<sub>4</sub>**,

i.e. a compound of formula I wherein

R<sub>1</sub> is a group (d),

R<sub>2</sub> is optionally protected hydroxy and there is a single bond in 23,24 position; or absent and there is a double bond in 23,24 position;

30 R<sub>4</sub> is hydroxy and there is a single bond in 10,11 position; and

R<sub>3</sub> is as defined in claim 1;

in free form or, where such forms exist, in salt form.

6. A process for the preparation of a compound according to claim 1 comprising

a) for the preparation of a compound of formula I wherein

35 R<sub>1</sub> is a group (a) as defined in claim 1,

R<sub>2</sub> and R<sub>3</sub> are as defined in claim 1 and

R<sub>4</sub> is hydroxy (i.e. a **compound Ia**),

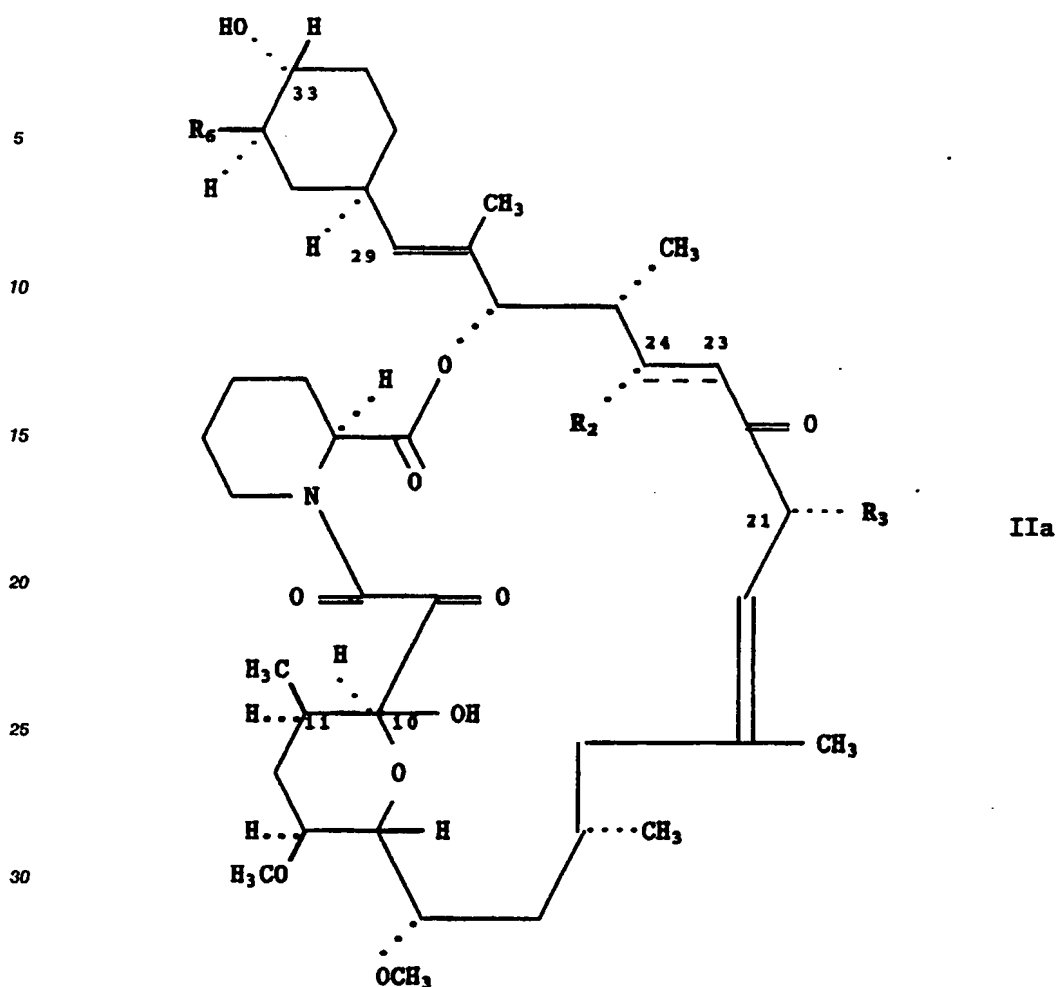
replacing under simultaneous epimerization the hydroxy group by chlorine, bromine, iodine or azido in a corresponding compound having unprotected hydroxy in 33 position (i.e. a **compound IIa**, of formula IIa

40

45

50

55



wherein  $R_2$  and  $R_3$  are as defined above under formula I and  $R_6$  is hydroxy or methoxy);

b) for the preparation of a compound of formula I wherein

$R_1$  is a group (b) as defined in claim 1,

$R_2$  and  $R_3$  are as defined in claim 1 and

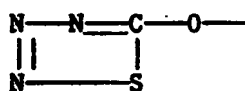
$R_4$  is hydroxy

(i.e. a compound Ib),

treating a corresponding compound IIa with cyanogen bromide in the presence of a base or

treating a corresponding compound IIa with thiophosgene, reacting the resultant product with an inorganic

azide and allowing the resultant unstable intermediate having a group



in 33 position (i.e. a compound IIb)

to decompose to a corresponding compound Ib;

c) for the preparation of a compound of formula I wherein

$R_1$  is a group (d) as defined in claim 1,

$R_2$  and  $R_3$  are as defined in claim 1 and

$R_4$  is hydroxy

(i.e. a compound Ic),

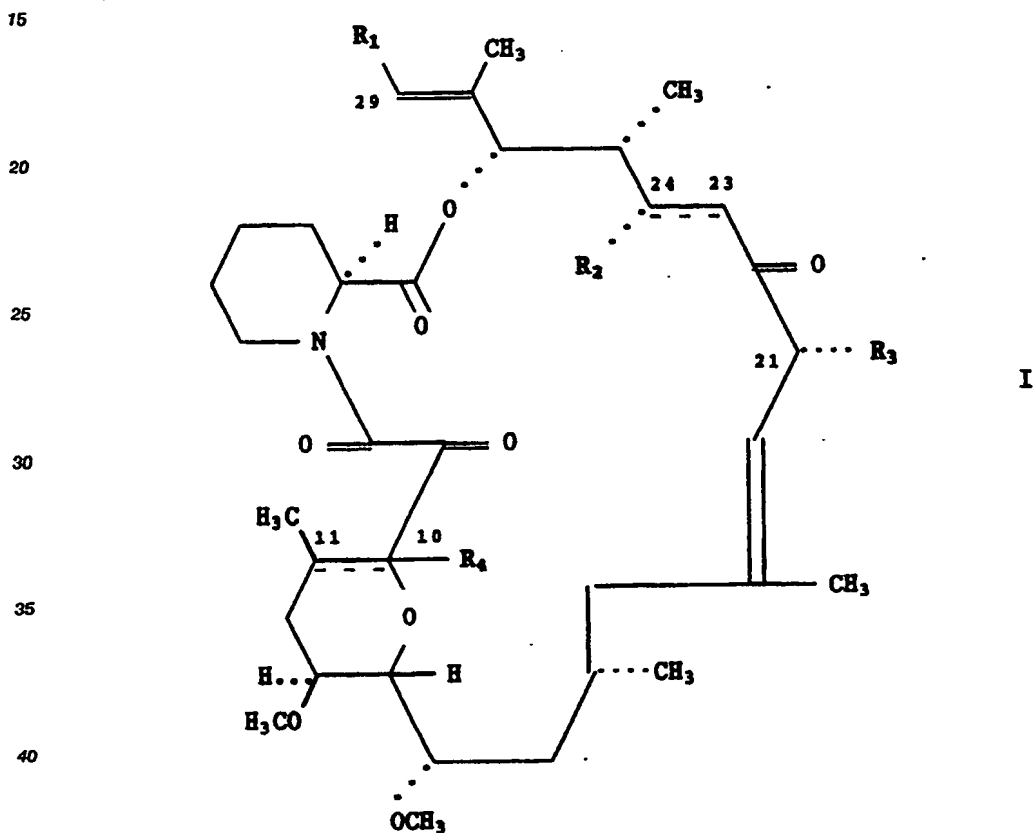
treating a corresponding compound Ib with an acid having a non-nucleophilic anion;

- d) for the preparation of a compound of formula I wherein  
 R<sub>1</sub> is a group (c) wherein R<sub>6</sub> is as defined in claim 1,  
 one of R<sub>2</sub> and R<sub>7</sub> is oxo or methylthiomethoxy and the other is protected hydroxy,  
 R<sub>3</sub> is as defined in claim 1 and  
 5 R<sub>4</sub> is hydroxy  
 (i.e. a compound Id),  
 treating a corresponding compound wherein  
 one of the substituents in 24 and 33 position is hydroxy and the other is protected hydroxy,  
 (i.e. a compound IIc)
- 10 with dimethylsulfoxide and acethanhydride;  
 e) for the preparation of a compound of formula I wherein  
 R<sub>1</sub> is a group (c) wherein  
 R<sub>6</sub> is as defined in claim 1 and  
 R<sub>7</sub> is isobutanoyloxy, aminooxalyloxy, R<sub>8</sub>R<sub>9</sub>CHCOO- as defined in claim 1 or p-tolyloxythiocarbonyloxy,  
 15 R<sub>2</sub> and R<sub>3</sub> are as defined in claim 1 and  
 R<sub>4</sub> is hydroxy  
 (i.e. a compound Ie),  
 appropriately acylating a corresponding compound IIa;  
 f) for the preparation of a compound of formula I wherein  
 20 R<sub>1</sub> is a group (c) wherein  
 R<sub>6</sub> is as defined in claim 1 and  
 R<sub>7</sub> is aminooxalyloxy,  
 R<sub>2</sub> is optionally protected hydroxy or is aminooxalyloxy,  
 R<sub>3</sub> is as defined in claim 1 and  
 25 R<sub>4</sub> is hydroxy  
 (i.e. a compound If),  
 treating with an appropriate oxalyl derivative and thereafter with ammonia a corresponding compound  
 having an optionally protected hydroxy group in 33 position and a protected hydroxy group in 24 position  
 (i.e. a compound IId);
- 30 g) for the preparation of a compound of formula I wherein  
 R<sub>1</sub> is a group (c) wherein R<sub>6</sub> is as defined in claim 1,  
 R<sub>2</sub> and R<sub>7</sub> are as defined in claim 1 with the proviso that one of R<sub>2</sub> and R<sub>7</sub> is methoxy,  
 R<sub>3</sub> is as defined in claim 1 and  
 R<sub>4</sub> is hydroxy  
 35 (i.e. a compound Ig),  
 methylating a corresponding compound having a hydroxy group in 24 or 33 position  
 (i.e. a compound IIe);  
 h) for the preparation of a compound of formula I wherein  
 R<sub>1</sub> is a group (c) wherein R<sub>6</sub> is as defined in claim 1,  
 40 R<sub>2</sub> and R<sub>7</sub> are as defined in claim 1 with the proviso that one of R<sub>2</sub> and R<sub>7</sub> is oxo,  
 R<sub>3</sub> is as defined in claim 1 and  
 R<sub>4</sub> is hydroxy  
 (i.e. a compound Ih),  
 oxidizing a corresponding compound having a hydroxy group in 24 or 33 position  
 45 (i.e. a compound IIf); and  
 - when a resultant compound of formula I has a protected hydroxy and/or a protected amino group,  
 optionally splitting off the protecting group(s) to give a corresponding compound of formula I having one or  
 more unprotected hydroxy and/or unprotected amino group(s)  
 (i.e. a compound IIj),
- 50 whereby when R<sub>1</sub> is a group (a), a water molecule may be simultaneously split off and a compound of  
 formula I is obtained wherein  
 R<sub>1</sub> is a group (a) as defined in claim 1,  
 R<sub>2</sub> is unprotected hydroxy and there is a single or double bond in 23,24 position; and  
 R<sub>4</sub> is absent and there is a double bond in 10,11 position (i.e. a compound II); or  
 55 - optionally protecting a unprotected hydroxy and/or unprotected amino group in a resultant compound of  
 formula I as appropriate to give a corresponding compound of formula I having one or more protected  
 hydroxy and/or protected amino groups(s) (i.e. a compound IIk);  
 and recovering the resultant compound of formula I in free form and, where such forms exist, in salt form.

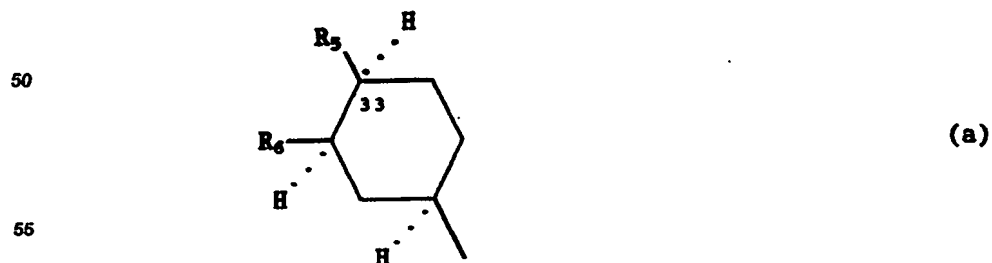
7. A pharmaceutical composition comprising a compound according to any one of claims 1 to 5 in free form or, where such forms exist, in pharmaceutically acceptable salt form, together with a pharmaceutically acceptable carrier or diluent.
8. A compound according to any one of claims 1 to 5 in free form or, where such forms exist, in pharmaceutically acceptable salt form, for use as a pharmaceutical.
9. A compound according to claim 8 for use in the preparation of a pharmaceutical composition by mixing with a pharmaceutically acceptable carrier or diluent.
10. A process for the preparation of a pharmaceutical composition comprising mixing a compound according to any one of claims 1 to 5 in free form or, where such forms exist, in pharmaceutically acceptable salt form, with a pharmaceutically acceptable carrier or diluent.

Claims for the following Contracting States: ES, GR

1. A process for the preparation of a compound of formula I



wherein  
either R<sub>1</sub> is a group (a) of formula



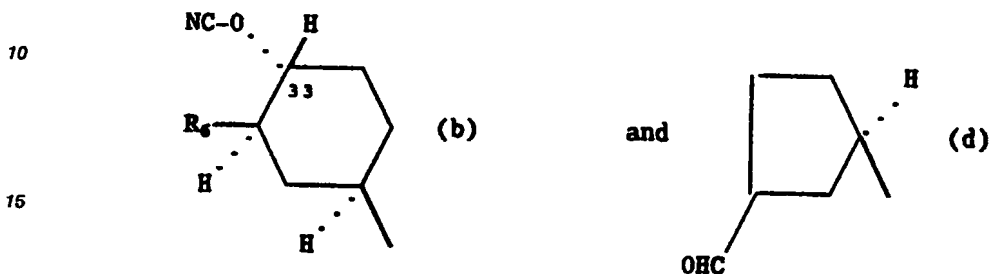
wherein  $R_5$  is chloro, bromo, iodo or azido and

$R_6$  is hydroxy or methoxy;

$R_2$  is oxo and there is a single bond in 23,24 position; optionally protected hydroxy and there is a single or a double bond in 23,24 position; or absent and there is a double bond in 23,24 position; and

5  $R_4$  is hydroxy and there is a single bond in 10,11 position; or absent and there is a double bond in 10,11 position;

or  $R_1$  is a group (b) or (d) of formula

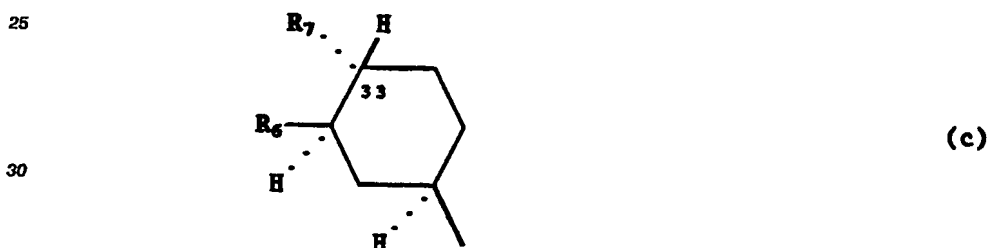


20 wherein  $R_6$  is as defined above;

$R_2$  is as defined above; and

$R_4$  is hydroxy and there is a single bond in 10,11 position;

or  $R_1$  is a group (c) of formula



35 wherein  $R_6$  is as defined above and

$R_7$  is oxo; optionally protected hydroxy; methoxy; methylthiomethoxy; isobutanoyloxy; aminooxalyloxy;  $R_8R_9CHCOO^-$  wherein  $R_8$  is optionally protected hydroxy or optionally protected amino and  $R_9$  is hydrogen or methyl; or p-tolyloxythiocarbonyloxy;

40  $R_2$  is oxo and there is a single bond in 23,24 position; absent and there is a double bond in 23,24 position; or is optionally protected hydroxy, methoxy, methylthiomethoxy, isobutanoyloxy, aminooxalyloxy or  $R_8R_9CHCOO^-$  wherein  $R_8$  and  $R_9$  are as defined above, and there is a single or a double bond in 23,24 position; whereby for group (c)

1) when  $R_7$  is oxo, unprotected hydroxy or methoxy

then  $R_2$  is other than absent and other than unprotected hydroxy or methoxy, and there is a single bond in 23,24 position;

45 2) when  $R_6$  is methoxy and  $R_7$  is methylthiomethoxy then  $R_2$  is other than absent and other than unprotected hydroxy;

3) when  $R_6$  is methoxy and  $R_7$  is protected hydroxy then  $R_2$  is other than optionally protected hydroxy; and

50 4) when  $R_6$  is hydroxy

then  $R_7$  is other than optionally protected hydroxy; and

$R_4$  is hydroxy and there is a single bond in 10,11 position; and  $R_3$  is methyl, ethyl, n-propyl or allyl; in free form or, where such forms exist, in salt form, comprising

55 a) for the preparation of a compound of formula I wherein

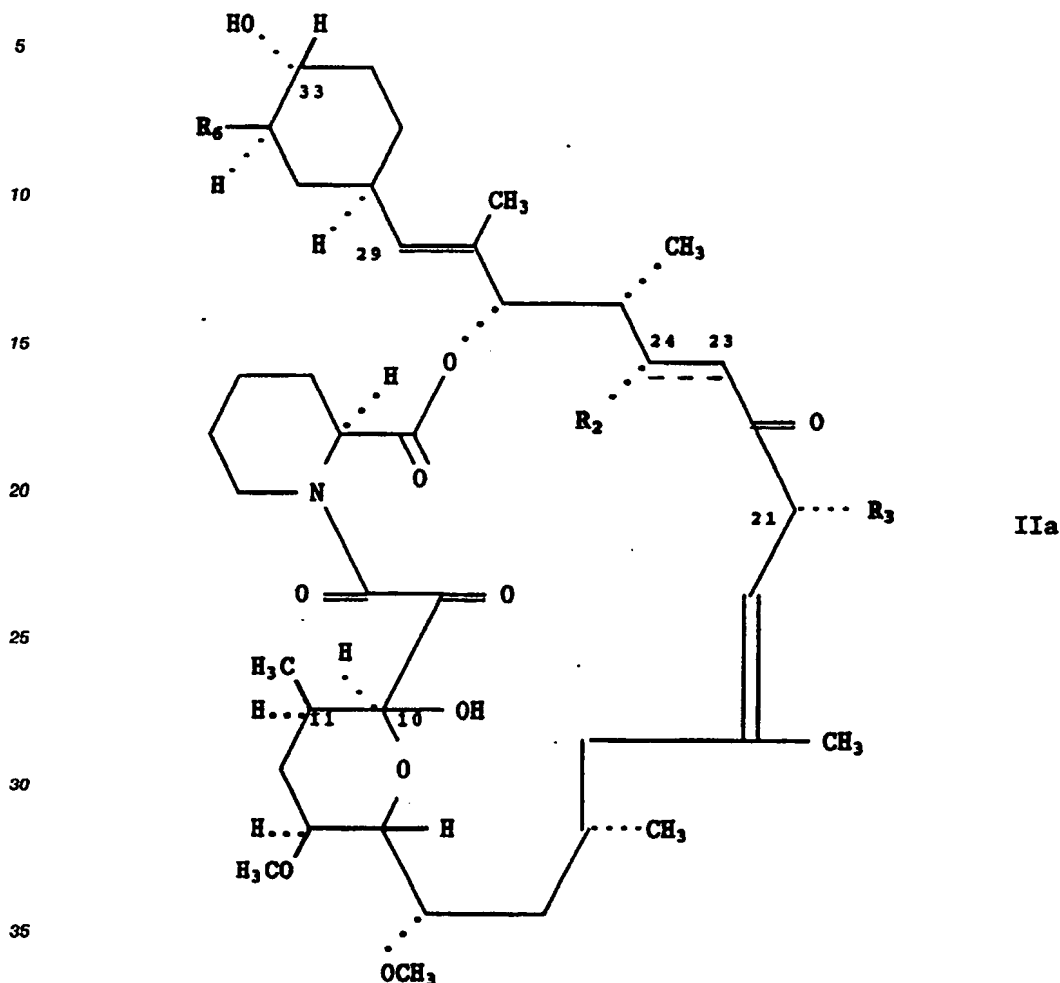
$R_1$  is a group (a) as defined in claim 1,

$R_2$  and  $R_3$  are as defined in claim 1 and

$R_4$  is hydroxy (i.e. a compound Ia),



replacing under simultaneous epimerization the hydroxy group by chlorine, bromine, iodine or azido in a corresponding compound having unprotected hydroxy in 33 position (i.e. a compound IIa, of formula IIa



wherein  $R_2$  and  $R_3$  are as defined above under formula I and  $R_6$  is hydroxy or methoxy);

b) for the preparation of a compound of formula I wherein

$R_1$  is a group (b) as defined in claim 1,

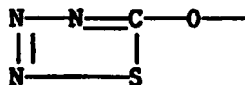
$R_2$  and  $R_3$  are as defined in claim 1 and

$R_4$  is hydroxy

(i.e. a compound Ib),

treating a corresponding compound IIa with cyanogen bromide in the presence of a base or

treating a corresponding compound IIa with thiophosgene, reacting the resultant product with an inorganic azide and allowing the resultant unstable intermediate having a group



in 33 position (i.e. a compound IIb)

to decompose to a corresponding compound Ib;

c) for the preparation of a compound of formula I wherein

$R_1$  is a group (d) as defined in claim 1,

$R_2$  and  $R_3$  are as defined in claim 1 and

- R<sub>4</sub> is hydroxy  
(i.e. a compound Ic),  
treating a corresponding compound Ib with an acid having a non-nucleophilic anion;  
d) for the preparation of a compound of formula I wherein
- 5 R<sub>1</sub> is a group (c) wherein R<sub>6</sub> is as defined in claim 1,  
one of R<sub>2</sub> and R<sub>7</sub> is oxo or methylthiomethoxy and the other is protected hydroxy,  
R<sub>3</sub> is as defined in claim 1 and  
R<sub>4</sub> is hydroxy  
(i.e. a compound Id),
- 10 treating a corresponding compound wherein  
one of the substituents in 24 and 33 position is hydroxy and the other is protected hydroxy,  
(i.e. a compound Ilc)  
with dimethylsulfoxide and acetanhydride;  
e) for the preparation of a compound of formula I wherein
- 15 R<sub>1</sub> is a group (c) wherein  
R<sub>6</sub> is as defined in claim 1 and  
R<sub>7</sub> is isobutanoyloxy, aminooxalyloxy, R<sub>8</sub>R<sub>9</sub>CHCOO- as defined in claim 1 or p-tolyloxythiocarbonyloxy,  
R<sub>2</sub> and R<sub>3</sub> are as defined in claim 1 and  
R<sub>4</sub> is hydroxy
- 20 (i.e. a compound Ie), appropriately acylating a corresponding compound IIa;  
f) for the preparation of a compound of formula I wherein  
R<sub>1</sub> is a group (c) wherein  
R<sub>6</sub> is as defined in claim 1 and  
R<sub>7</sub> is aminooxalyloxy,
- 25 R<sub>2</sub> is optionally protected hydroxy or is aminooxalyloxy,  
R<sub>3</sub> is as defined in claim 1 and  
R<sub>4</sub> is hydroxy  
(i.e. a compound If),  
treating with an appropriate oxalyl derivative and thereafter with ammonia a corresponding compound
- 30 having an optionally protected hydroxy group in 33 position and a protected hydroxy group in 24 position  
(i.e. a compound IId);  
g) for the preparation of a compound of formula I wherein  
R<sub>1</sub> is a group (c) wherein R<sub>6</sub> is as defined in claim 1,  
R<sub>2</sub> and R<sub>7</sub> are as defined in claim 1 with the proviso that one of R<sub>2</sub> and R<sub>7</sub> is methoxy,
- 35 R<sub>3</sub> is as defined in claim 1 and  
R<sub>4</sub> is hydroxy  
(i.e. a compound Ig),  
methylating a corresponding compound having a hydroxy group in 24 or 33 position  
(i.e. a compound IIf);
- 40 h) for the preparation of a compound of formula I wherein  
R<sub>1</sub> is a group (c) wherein R<sub>6</sub> is as defined in claim 1,  
R<sub>2</sub> and R<sub>7</sub> are as defined in claim 1 with the proviso that one of R<sub>2</sub> and R<sub>7</sub> is oxo,  
R<sub>3</sub> is as defined in claim 1 and  
R<sub>4</sub> is hydroxy
- 45 (i.e. a compound Ih),  
oxidizing a corresponding compound having a hydroxy group in 24 or 33 position  
(i.e. a compound IIg); and  
- when a resultant compound of formula I has a protected hydroxy and/or a protected amino group,  
optionally splitting off the protecting group(s) to give a corresponding compound of formula I having one or
- 50 more unprotected hydroxy and/or unprotected amino group(s)  
(i.e. a compound IIj),  
whereby when R<sub>1</sub> is a group (a), a water molecule may be simultaneously split off and a compound of  
formula I is obtained wherein  
R<sub>1</sub> is a group (a) as defined in claim 1,
- 55 R<sub>2</sub> is unprotected hydroxy and there is a single or double bond in 23,24 position; and  
R<sub>4</sub> is absent and there is a double bond in 10,11 position (i.e. a compound IIk); or  
- optionally protecting an unprotected hydroxy and/or unprotected amino group in a resultant compound of  
formula I as appropriate to give a corresponding compound of formula I having one or more protected

hydroxy and/or protected amino groups(s) (i.e. a compound Ik);

and recovering the resultant compound of formula I in free form and, where such forms exist, in salt form.

2. A process according to claim 1 for the preparation of the compound according to claim 1 which is 29-des-(4-hydroxy-3-methoxycyclohexyl)-29-(3-formylcyclopentyl)-FR 520 (compound of Example 12).
- 5 3. A process according to claim 1 for the preparation of the compound according to claim 1 which is 33-aminooxalyloxy-FR 520 (compound of Example 28a) in free form or in salt form.
4. A process according to claim 1 for the preparation of the compound according to claim 1 which is FR 520-33-glycolate (compound of Examples 32 and 54).
5. A process according to claim 1 for the preparation of the compound according to claim 1 which is 33-isobutanoyloxy-FR 520 (compound of Examples 37 and 62).
- 10 6. A process according to claim 1 for the preparation of the compound according to claim 1 which is 33-epi-33-chloro-FR 520 (compound of Example 66a).
7. A process for the preparation of a pharmaceutical composition comprising mixing a compound of formula I as defined in claim 1 in free form or, where such forms exist, in pharmaceutically acceptable salt form,
- 15 with a pharmaceutically acceptable carrier or diluent.

20

25

30

35

40

45

50

55



European Patent  
Office

# EUROPEAN SEARCH REPORT

Application Number

DOCUMENTS CONSIDERED TO BE RELEVANT			EP 90810854.1
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
X	JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, vol. 109, 1987 H. TANAKA et al. "Structure of FK 506: "A novel immunosuppressant isolated from Streptomyces" pages 5031-5033 * Page 5031 *	1,7-10	C 07 D 498/18 A 61 K 31/33 //(C 07 D 498/18 C 07 D 311:00 C 07 D 273:00 C 07 D 221:00)
A	EP - A2 - 0 184 162 (FUJISAWA PHARM.CO.LTD.) * Claims 1-18 *	1-10	
A	EP - A2 - 0 227 355 (THE UNIVERSITY OF KANSAS) * Pages 2-4; claims *	1-10	
A	EP - A1 - 0 323 042 (FISONS PLC.) * Pages 3,7; claims *	1-8	
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)
			C 07 D 498/00
The present search report has been drawn up for all claims			
Place of search WIEN		Date of completion of the search 17-01-1991	Examiner JANISCH
CATEGORY OF CITED DOCUMENTS			
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		I : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons A : member of the same patent family, corresponding document	